

PATENT APPLICATION
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for

INTEGRATED MONOLITHIC MICROFABRICATED ELECTROSPRAY AND LIQUID CHROMATOGRAPHY SYSTEM AND METHOD

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3 FIELD OF THE INVENTION

4 The present invention relates generally to an integrated miniaturized chemical
5 analysis system fabricated using microelectromechanical systems (MEMS) technology. In
6 particular, the present invention relates to an integrated monolithic microfabricated
7 electrospray and liquid chromatography device. This achieves a significant advantage in
8 terms of high-throughput analysis by mass spectrometry, as used, for example, in drug
9 discovery, in comparison to a conventional system.

10 BACKGROUND OF THE INVENTION

11 New developments in drug discovery and development are creating new demands
12 on analytical techniques. For example, combinatorial chemistry is often employed to
13 discover new lead compounds. or to create variations of a lead compound. Combinatorial
14 chemistry techniques can generate thousands or millions of compounds (combinatorial
15 libraries) in a relatively short time (on the order of days to weeks). Testing such a large
16 number of compounds for biological activity in a timely and efficient manner requires high-
17 throughput screening methods which allow rapid evaluation of the characteristics of each
18 candidate compound.

19 The compounds in combinatorial libraries are often tested simultaneously against
20 a molecular target. For example, an enzyme assay employing a colorimetric measurement
21 may be run in a 96-well plate. An aliquot of enzyme in each well is combined with tens or
22 hundreds of compounds. An effective enzyme inhibitor will prevent development of color
23 due to the normal enzyme reaction, allowing for rapid spectroscopic (or visual) evaluation
24 of assay results. If ten compounds are present in each well, 960 compounds can be screened
25 in the entire plate, and one hundred thousand compounds can be screened in 105 plates,
26 allowing for rapid and automated testing of the compounds.

Often, however, determination of which compounds are present in certain portions
of a combinatorial library is difficult, due to the manner of synthesis of the library. For
example, the "split-and-pool" method of random peptide synthesis in U.S. Pat. No.
5,182,366, describes a way of creating a peptide library where each resin bead carries a
unique peptide sequence. Placing ten beads in each well of a 96-well plate, followed by
cleavage of the peptides from the beads and removal of the cleavage solution, would result

1 in ten (or fewer) peptides in each well of the plate. Enzyme assays could then be carried out
2 in the plate wells, allowing 100,000 peptides to be screened in 105 plates. However, the
identity of the peptides would not be known, requiring analysis of the contents of each well.

3 The peptides could be analyzed by removing a portion of solution from each well
4 and injecting the contents into a separation device such as liquid chromatography or capillary
5 electrophoresis instrument coupled to a mass spectrometer. Assuming that such a method
6 would take approximately 5 minutes per analysis, it would require over a month to analyze
the contents of 105 96-well plates, assuming the method was fully automated and operating
7 24 hours a day.

8 This example illustrates the critical need for a method for rapid analysis of large
9 numbers of compounds or complex mixtures of compounds, particularly in the context of
high-throughput screening. Techniques for generating large numbers of compounds, for
10 example through combinatorial chemistry, have been established. High-throughput
screening methods are under development for a wide variety of targets, and some types of
11 screens, such as the colorimetric enzyme assay described above and ELISA (enzyme linked
12 immunosorbent assay) technology, are well-established. As indicated in the example above,
13 a bottleneck often occurs at the stage where multiple mixtures of compounds, or even
multiple individual compounds, must be characterized.

14 This need is further underscored when current developments in molecular
15 biotechnology are considered. Enormous amounts of genetic sequence data are being
16 generated through new DNA sequencing methods. This wealth of new information is
generating new insights into the mechanism of disease processes. In particular, the
17 burgeoning field of genomics has allowed rapid identification of new targets for drug
development efforts. Determination of genetic variations between individuals has opened
18 up the possibility of targeting drugs to individuals based on the individual's particular genetic
19 profile. Testing for cytotoxicity, specificity, and other pharmaceutical characteristics could
20 be carried out in high-throughput assays instead of expensive animal testing and clinical
21 trials. Detailed characterization of a potential drug or lead compound early in the drug
development process thus has the potential for significant savings both in time and expense.

22 Development of viable screening methods for these new targets will often depend
23 on the availability of rapid separation and analysis techniques for analyzing the results of
24 assays. For example, an assay for potential toxic metabolites of a candidate drug would need
25 to identify both the candidate drug and the metabolites of that candidate. An assay for
26

1 specificity would need to identify compounds which bind differentially to two molecular
2 targets such as a viral protease and a mammalian protease.

3 It would therefore be advantageous to provide a method for efficient proteomic
4 screening in order to obtain the pharmacokinetic profile of a drug early in the evaluation
5 process. An understanding of how a new compound is absorbed in the body and how it is
6 metabolized can enable prediction of the likelihood for an increased therapeutic effect or lack
7 thereof.

8 Given the enormous number of new compounds that are being generated daily, an
9 improved system for identifying molecules of potential therapeutic value for drug discovery
10 is also critically needed.

11 It also would be desirable to provide rapid sequential analysis and identification of
12 compounds which interact with a gene or gene product that plays a role in a disease of
13 interest. Rapid sequential analysis can overcome the bottleneck of inefficient and time-
14 consuming serial (one-by-one) analysis of compounds.

15 Accordingly, there is a critical need for high-throughput screening and identification
16 of compound-target reactions in order to identify potential drug candidates.

17 Microchip-based separation devices have been developed for rapid analysis of large
18 numbers of samples. Compared to other conventional separation devices, these microchip-
19 based separation devices have higher sample throughput, reduced sample and reagent
20 consumption and reduced chemical waste. The liquid flow rates for microchip-based
21 separation devices range from approximately 1-300 nanoliters (nL) per minute for most
22 applications.

23 Examples of microchip-based separation devices include those for capillary
24 electrophoresis (CE), capillary electrochromatography (CEC) and high-performance liquid
25 chromatography (HPLC). See Harrison *et al*, Science 1993, 261, 859-897; Jacobson *et al*.
26 Anal. Chem. 1994, 66, 1114-1118; and Jacobson *et al*. Anal. Chem. 1994, 66, 2369-2373.
Such separation devices are capable of fast analyses and provide improved precision and
reliability compared to other conventional analytical instruments.

Liquid chromatography (LC) is a well-established analytical method for separating
components of a fluid for subsequent analysis and/or identification. Traditionally, liquid
chromatography utilizes a separation column, such as a cylindrical tube, filled with tightly
packed beads, gel or other appropriate particulate material to provide a large surface area.
The large surface area facilitates fluid interactions with the particulate material, and the
tightly packed, random spacing of the particulate material forces the liquid to travel over a

1 much longer effective path than the length of the column. In particular, the components of
2 the fluid interact with the stationary phase (the particles in the liquid chromatography
3 column) as well as the mobile phase (the liquid eluent flowing through the liquid
4 chromatography column) based on the partition coefficients for each of the components. The
5 partition coefficient is defined as the ratio of the time an analyte spends interacting with
6 the stationary phase to the time spent interacting with the mobile phase. The longer an
7 analyte interacts with the stationary phase, the higher the partition coefficient and the longer
8 the analyte is retained on the liquid chromatography column. The components may be
9 detected spectroscopically after elution from the liquid chromatography column by coupling
10 the exit of the column to a post-column detector.

11 Spectroscopic detectors rely on a change in refractive index, ultraviolet and/or
12 visible light absorption, or fluorescence after excitation with a suitable wavelength to detect
13 the separated components. Alternatively, the separated components may be passed from the
14 liquid chromatography column into other types of analytical instruments for analysis. The
15 analysis outcome depends upon the sequenced arrival of the components separated by the
16 liquid chromatography column and is therefore time-dependent.

17 The length of liquid transport from the liquid chromatography column to the analysis
18 instrument such as the detector is preferably minimized in order to minimize diffusion and
19 thereby maximize the separation efficiency and analysis sensitivity. The transport length is
20 referred to as the dead volume or extra-column volume.

21 Capillary electrophoresis is a technique that utilizes the electrophoretic nature of
22 molecules and/or the electroosmotic flow of fluids in small capillary tubes to separate
23 components of a fluid. Typically a fused silica capillary of 100 μm inner diameter or less is
24 filled with a buffer solution containing an electrolyte. Each end of the capillary is placed in
25 a separate fluidic reservoir containing a buffer electrolyte.

26 A potential voltage is placed in one of the buffer reservoirs and a second potential
voltage is placed in the other buffer reservoir. Positively and negatively charged species will
migrate in opposite directions through the capillary under the influence of the electric field
established by the two potential voltages applied to the buffer reservoirs. Electroosmotic
flow is defined as the fluid flow along the walls of a capillary due to the migration of charged
species from the buffer solution. Some molecules exist as charged species when in solution
and will migrate through the capillary based on the charge-to-mass ratio of the molecular
species. This migration is defined as electrophoretic mobility. The electroosmotic flow and
the electrophoretic mobility of each component of a fluid determine the overall migration for

1 each fluidic component. The fluid flow profile resulting from electroosmotic flow is flat due
2 to the reduction in frictional drag along the walls of the separation channel. This results in
3 improved separation efficiency over liquid chromatography where the flow profile is
4 parabolic resulting from pressure driven flow.

5 Capillary electrochromatography is a hybrid technique which utilizes the electrically
6 driven flow characteristics of electrophoretic separation methods within capillary columns
7 packed with a solid stationary phase typical of liquid chromatography. It couples the
8 separation power of reversed-phase liquid chromatography with the high efficiencies of
9 capillary electrophoresis. Higher efficiencies are obtainable for capillary electro-
10 chromatography separations over liquid chromatography because the flow profile resulting
11 from electroosmotic flow is flat due to the reduction in frictional drag along the walls of the
12 separation channel when compared to the parabolic flow profile resulting from pressure
13 driven flows. Furthermore, smaller particle sizes can be used in capillary
14 electrochromatography than in liquid chromatography because no back pressure is generated
15 by electroosmotic flow. In contrast to electrophoresis, capillary electrochromatography is
16 capable of separating neutral molecules due to analyte partitioning between the stationary and
17 mobile phases of the column particles using a liquid chromatography separation mechanism.

18 The separated product of such separation devices may be introduced as the liquid
19 sample to a device that is used to produce electrospray ionization. The electrospray device
20 may be interfaced to an atmospheric pressure ionization mass spectrometer (API-MS) for
21 analysis of the electrosprayed fluid.

22 A schematic of an electrospray system 50 is shown in FIG. 1. An electrospray is
23 produced when a sufficient electrical potential difference V_{spray} is applied between a
24 conductive or partly conductive fluid exiting a capillary orifice and an electrode so as to
25 generate a concentration of electric field lines emanating from the tip or end of a capillary
26 52 of an electrospray device. When a positive voltage V_{spray} is applied to the tip of the
capillary relative to an extracting electrode 54, such as one provided at the ion-sampling
orifice to the mass spectrometer, the electric field causes positively-charged ions in the fluid
to migrate to the surface of the fluid at the tip of the capillary. When a negative voltage V_{spray}
is applied to the tip of the capillary relative to an extracting electrode 54, such as one
provided at the ion-sampling orifice to the mass spectrometer, the electric field causes
negatively-charged ions in the fluid to migrate to the surface of the fluid at the tip of the
capillary.

1 When the repulsion force of the solvated ions exceeds the surface tension of the fluid
2 sample being electrosprayed, a volume of the fluid sample is pulled into the shape of a cone,
3 known as a Taylor cone 56 which extends from the tip of the capillary. Small charged
4 droplets 58 are formed from the tip of the Taylor cone 56 and are drawn toward the
5 extracting electrode 54. This phenomenon has been described, for example, by Dole et al.,
6 *Chem. Phys.* 1968, 49, 2240 and Yamashita and Fenn, *J. Phys. Chem.* 1984, 88, 4451. The
7 potential voltage required to initiate an electrospray is dependent on the surface tension of
8 the solution as described by, for example, Smith, *IEEE Trans. Ind. App.* 1986, IA-22, 527-
9 535. Typically, the electric field is on the order of approximately 10^6 V/m. The physical
10 size of the capillary determines the density of electric field lines necessary to induce
11 electrospray.

12 One advantage of electrospray ionization is that the response for an analyte measured
13 by the mass spectrometer detector is dependent on the concentration of the analyte in the
14 fluid and independent of the fluid flow rate. The response of an analyte in solution at a given
15 concentration would be comparable using electrospray ionization combined with mass
16 spectrometry at a flow rate of 100 μ L/min compared to a flow rate of 100 nL/min.

17 The process of electrospray ionization at flow rates on the order of nanoliters per
18 minute has been referred to as "nanoelectrospray". Electrospray into the ion-sampling orifice
19 of an API mass spectrometer produces a quantitative response from the mass spectrometer
20 detector due to the analyte molecules present in the liquid flowing from the capillary.

21 Thus, it is desirable to provide an electrospray ionization device for integration
22 upstream with microchip-based separation devices and for integration downstream with API-
23 MS instruments.

24 Attempts have been made to manufacture an electrospray device which produces
25 nanoelectrospray. For example, Wilm and Mann, *Anal. Chem.* 1996, 68, 1-8 describes the
26 process of electrospray from fused silica capillaries drawn to an inner diameter of 2-4 μ m at
flow rates of 20 nL/min. Specifically, a nanoelectrospray at 20 nL/min was achieved from
a 2 μ m inner diameter and 5 μ m outer diameter pulled fused-silica capillary with 600-700
V at a distance of 1-2 mm from the ion-sampling orifice of an API mass spectrometer.

Ramsey et al., *Anal. Chem.* 1997, 69, 1174-1178 describes nanoelectrospray at 90
nL/min from the edge of a planar glass microchip with a closed separation channel 10 μ m
deep, 60 μ m wide and 33 mm in length using electroosmotic flow and applying 4.8 kV to the
fluid exiting the closed separation channel on the edge of the microchip for electrospray
formation, with the edge of the chip at a distance of 3-5 mm from the ion-sampling orifice

1 of an API mass spectrometer. Approximately 12 nL of the sample fluid collects at the edge
2 of the chip before the formation of a Taylor cone and stable nanoelectrospray from the edge
3 of the microchip. However, collection of approximately 12 nL of the sample fluid will result
4 in remixing of the fluid, thereby undoing the separation done in the separation channel.
5 Remixing causes band broadening at the edge of the microchip, fundamentally limiting its
6 applicability for nanoelectrospray-mass spectrometry for analyte detection. Thus,
7 nanoelectrospray from the edge of this microchip device after capillary electrophoresis or
8 capillary electrochromatography separation is rendered impractical. Furthermore, because
9 this device provides a flat surface, and thus a relatively small amount of physical asperity,
10 for the formation of the electrospray, the device requires an impractically high voltage to
11 initiate electrospray, due to poor field line concentration.

12 Xue, Q.; Foret, F.; Dunayevskiy, Y. M.; Zavracky, P. M.; McGruer, N.E.; Karger,
13 B. L. *Anal. Chem.* 1997, 69, 426-430 describes a stable nanoelectrospray from the edge of
14 a planar glass microchip with a closed channel 25 μm deep, 60 μm wide and 35-50 mm in
15 length and applying 4.2 kV to the fluid exiting the closed separation channel on the edge of
16 the microchip for electrospray formation, with the edge of the chip at a distance of 3-8 mm
17 from the ion-sampling orifice of an API mass spectrometer. A syringe pump is utilized to
18 deliver the sample fluid to the glass microchip electrosprayer at a flow rate between 100-200
19 nL/min. The edge of the glass microchip is treated with a hydrophobic coating to alleviate
20 some of the difficulties associated with nanoelectrospray from a flat surface and which
21 slightly improves the stability of the nanoelectrospray. Electrospraying in this manner from
22 a flat surface again results in poor field line concentration and yields an inefficient
23 electrospray.

24 Desai et al. 1997 *International Conference on Solid-State Sensors and Actuator*,
25 Chicago, June 16-19, 1997, 927-930 describes a multi-step process to generate a nozzle on
26 the edge of a silicon microchip 1-3 μm in diameter or width and 40 μm in length and
applying 4 kV to the entire microchip at a distance of 0.25-0.4 mm from the ion-sampling
orifice of an API mass spectrometer. This nanoelectrospray nozzle reduces the dead volume
of the sample fluid. However, the extension of the nozzle from the edge of the microchip
exposes the nozzle to accidental breakage. Because a relatively high spray voltage was
utilized and the nozzle was positioned in very close proximity to the mass spectrometer
sampling orifice, a poor field line concentration and a low efficient electrospray were
achieved.

In all of the above-described devices, edge-spraying from a monolithic chip is a

1 poorly controlled process due to the inability to rigorously and repeatably determine the
2 physical form of the chip's edge. In another embodiment of edge-spraying, ejection nozzles,
3 such as small segments of drawn capillaries, are separately and individually attached to the
4 chip's edge. This process is inherently cost-inefficient and unreliable, imposes space
5 constraints in chip design, and is therefore unsuitable for manufacturing.

6 Thus, it is also desirable to provide an electrospray ionization device with
7 controllable spraying and a method for producing such a device which is easily reproducible
8 and manufacturable in high volumes.

9 SUMMARY OF THE INVENTION

10 The present invention provides a silicon microchip-based electrospray device for
11 producing reproducible, controllable and robust nanoelectrospray ionization of a liquid
12 sample. The electrospray device may be interfaced downstream to an atmospheric pressure
13 ionization mass spectrometer (API-MS) for analysis of the electrosprayed fluid and/or
14 interfaced upstream to a miniaturized liquid phase separation device, which may have, for
15 example, glass, plastic or silicon substrates or wafers.

16 The electrospray device of the present invention generally comprises a silicon
17 substrate or microchip defining a channel between an entrance orifice on an injection surface
18 and a nozzle on an ejection surface (the major surface) such that the electrospray generated
19 by the electrospray device is generally approximately perpendicular to the ejection surface.
20 The nozzle has an inner and an outer diameter and is defined by an annular portion recessed
21 from the ejection surface. The annular recess extends radially from the outer diameter. The
22 tip of the nozzle is co-planar or level with and does not extend beyond the ejection surface
23 and thus the nozzle is protected against accidental breakage. The nozzle, channel and
24 recessed portion are etched from the silicon substrate by reactive-ion etching and other
25 standard semiconductor processing techniques.

26 All surfaces of the silicon substrate preferably have a layer of silicon dioxide thereon
created by oxidization to electrically isolate the liquid sample from the substrate and the
ejection and injection surfaces from each other such that different potential voltages may be
individually applied to each surface and the liquid sample. The silicon dioxide layer also
provides for biocompatibility. The electrospray apparatus further comprises at least one
controlling electrode electrically contacting the substrate through the oxide layer for the
application of an electric potential to the substrate.

1 Preferably, the nozzle, channel and recess are etched from the silicon substrate by
2 reactive-ion etching and other standard semiconductor processing techniques. The injection-
3 side feature(s), through-substrate fluid channel, ejection-side features, and controlling
4 electrodes - are formed monolithically from a monocrystalline silicon substrate. That is, they
5 are formed during the course of and as a result of a fabrication sequence that requires no
6 manipulation or assembly of separate components.

7 Because the electrospray device is manufactured using reactive-ion etching and other
8 standard semiconductor processing techniques, the dimensions of such a device can be very
9 small, for example, as small as 2 μm inner diameter and 5 μm outer diameter. Thus, a nozzle
10 having, for example, 5 μm inner diameter and 250 μm in height only has a volume of 4.9 pL
11 (picoliter). In contrast, an electrospray device from the flat edge of a glass microchip would
12 introduce additional dead volume of 12 nL compared to the volume of a separation channel
13 of 19.8 nL thereby allowing remixing of the fluid components and undoing the separation
14 done by the separation channel. The micrometer-scale dimensions of the electrospray device
15 minimizes the dead volume and thereby increases efficiency and analysis sensitivity.

16 The electrospray device of the present invention provides for the efficient and
17 effective formation of an electrospray. By providing an electrospray surface from which the
18 fluid is ejected with dimensions on the order of micrometers, the electrospray device limits
19 the voltage required to generate a Taylor cone as the voltage is dependent upon the nozzle
20 diameter, surface tension of the fluid and the distance of the nozzle from the extracting
21 electrode. The nozzle of the electrospray device provides the physical asperity on the order
22 of micrometers on which a large electric field is concentrated. Further, the electrospray
23 device may provide additional electrode(s) on the ejecting surface to which electric
24 potential(s) may be applied and controlled independent of the electric potentials of the fluid
25 and the extracting electrode in order to advantageously modify and optimize the electric
26 field. The combination of the nozzle and the additional electrode(s) thus enhance the electric
27 field between the nozzle and the extracting electrode. The large electric field, on the order
28 of 10^6 V/m or greater and generated by the potential difference between the fluid and
29 extracting electrode, is thus applied directly to the fluidic cone rather than uniformly
30 distributed in space.

31 The microchip-based electrospray ionization device of the present invention
32 provides minimal extra-column dispersion as a result of a reduction in the extra-column
33 volume and provides efficient, reproducible, reliable and rugged formation of an
34 electrospray. The design of the ionization device is also robust such that the electrospray

1 device can be readily mass-produced in a cost-effective, high-yielding process.

2 In operation, a conductive or partly conductive liquid sample is introduced into the
3 channel through the entrance orifice on the injection surface. The liquid sample and nozzle
4 are held at the potential voltage applied to the fluid, either by means of a wire within the fluid
5 delivery channel to the electrospray device or by means of an electrode formed on the
6 injection surface isolated from the surrounding surface region and from the substrate. The
7 electric field strength at the tip of the nozzle is enhanced by the application of a voltage to
8 the substrate and/or the ejection surface, preferably approximately less than one-half of the
9 voltage applied to the fluid. Thus, by the independent control of the fluid/nozzle and
10 substrate/ejection surface voltages, the electrospray device of the present invention allows
11 the optimization of the electric field lines emanating from the nozzle. Further, when the
12 electrospray device is interfaced downstream with a mass spectrometry device, the
13 independent control of the fluid/nozzle and substrate/ejection surface voltages also allows
14 for the direction and optimization of the electrospray into an acceptance region of the mass
15 spectrometry device.

16 The electrospray device of the present invention may be placed 1-2 mm or up to 10
17 mm from the orifice of an API mass spectrometer to establish a stable nanoelectrospray at
18 flow rates as low as 20 nL/min with a voltage of, for example, 700 V applied to the nozzle
19 and 0-350 V applied to the substrate and/or the planar ejection surface of the silicon
20 microchip.

21 An array or matrix of multiple electrospray devices of the present invention may be
22 manufactured on a single microchip as silicon fabrication using standard, well-controlled
23 thin-film processes not only eliminates handling of such micro components but also allows
24 for rapid parallel processing of functionally alike elements. The nozzles may be radially
25 positioned about a circle having a relatively small diameter near the center of the chip. Thus,
26 the electrospray device of the present invention provides significant advantages of time and
cost efficiency, control, and reproducibility. The low cost of these electrospray devices
allows for one-time use such that cross-contamination from different liquid samples may be
eliminated.

The electrospray device of the present invention can be integrated upstream with
miniaturized liquid sample handling devices and integrated downstream with an API mass
spectrometer. The electrospray device may be chip-to-chip or wafer-to-wafer bonded to
silicon microchip-based liquid separation devices capable of, for example, capillary
electrophoresis, capillary electrochromatography, affinity chromatography, liquid

1 chromatography (LC) or any other condensed-phase separation technique. The electrospray
2 device may be alternatively bonded to glass-and/or polymer-based liquid separation devices
3 with any suitable method.

4 In another aspect of the invention, a microchip-based liquid chromatography
5 device may be provided. The liquid chromatography device generally comprises a separation
6 substrate or wafer defining an introduction channel between an entrance orifice and a
7 reservoir and a separation channel between the reservoir and an exit orifice. The separation
8 channel is populated with separation posts extending from a side wall of the separation
9 channel perpendicular to the fluid flow through the separation channel. Preferably, the
10 separation posts do not extend beyond and are preferably coplanar or level with the surface
11 of the separation substrate such that they are protected against accidental breakage during the
12 manufacturing process. Component separation occurs in the separation channel where the
13 separation posts perform the liquid chromatography function by providing large surface areas
14 for the interaction of fluid flowing through the separation channel. A cover substrate may
15 be bonded to the separation substrate to enclose the reservoir and the separation channel
16 adjacent the cover substrate.

17 The liquid chromatography device may further comprise one or more electrodes for
18 application of electric potentials to the fluid at locations along the fluid path. The application
19 of different electric potentials along the fluid path may facilitate the fluid flow through the
20 fluid path.

21 The introduction and separation channels, the entrance and exit orifices and the
22 separation posts are preferably etched from a silicon substrate by reactive-ion etching and
23 other standard semiconductor processing techniques. The separation posts are preferably
24 oxidized silicon posts which may be chemically modified to optimize the interaction of the
25 components of the sample fluid with the stationary separation posts.

26 In another aspect of the invention, the liquid chromatography device may be
integrated with the electrospray device such that the exit orifice of the liquid chromatography
device forms a homogenous interface with the entrance orifice of the electrospray device,
thereby allowing the on-chip delivery of fluid from the liquid chromatography device to the
electrospray device to generate an electrospray. The nozzle, channel and recessed portion
of the electrospray device may be etched from the cover substrate of the liquid
chromatography device.

In yet another aspect of the invention, multiples of the liquid chromatography-
electrospray system may be formed on a single chip to deliver a multiplicity of samples to

1 a common point for subsequent sequential analysis. The multiple nozzles of the electrospray
2 devices may be radially positioned about a circle having a relatively small diameter near the
center of the single chip.

3 The radially distributed array of electrospray nozzles on a multi-system chip may be
4 interfaced with a sampling orifice of a mass spectrometer by positioning the nozzles near the
5 sample orifice. The tight radial configuration of the electrospray nozzles allows the
6 positioning thereof in close proximity to the sampling orifice of a mass spectrometer.

7 The multi-system chip thus provides a rapid sequential chemical analysis system
8 fabricated using microelectromechanical systems (MEMS) technology. For example, the
9 multi-system chip enables automated, sequential separation and injection of a multiplicity
10 of samples, resulting in significantly greater analysis throughput and utilization of the mass
spectrometer instrument for, for example, high-throughput detection of compounds for drug
discovery.

11 BRIEF DESCRIPTION OF THE DRAWINGS

12 The file of this patent contains at least one drawing executed in color. Copies of this
13 patent with color drawings will be provided by the Patent and Trademark Office upon request
14 and payment of the necessary fee.

15 FIG. 1 shows a schematic of an electrospray system;

16 FIG. 2 shows a perspective view of an electrospray device of the present invention;

17 FIG. 3 shows a plan view of the electrospray device of FIG. 2;

18 FIG. 4 shows a cross-sectional view of the electrospray device of FIG. 3 taken along
line 4-4;

19 FIG. 5 shows a schematic of an electrospray system comprising an electrospray
device of the present invention;

20 FIG. 6 shows a plan view of an electrospray device having multiple electrodes on
the ejection surface of the device;

21 FIG. 7 shows a cross-sectional view of the electrospray device of FIG. 6 taken along
22 line 7-7;

23 FIG. 8 illustrates a feedback control circuit incorporating an electrospray device of
the present invention;

24 FIGS. 9-20G show an example of a fabrication sequence of the electrospray device;

1 FIG. 21 A shows a cross-sectional view of a piezoelectric pipette positioned at a
2 distance from and for delivery of a fluid sample to the entrance orifice of the electrospray
device;

3 FIG. 21 B shows a cross-sectional view of a capillary for delivery of a fluid sample
4 to and prior to attachment to the entrance orifice of the electrospray device;

5 FIG. 22 shows a schematic of a single integrated system comprising an upstream
6 fluid delivery device and an electrospray device having a homogeneous interface with the
fluid delivery device;

7 FIG. 23A shows an exploded perspective view of a chip-based combinatorial
8 chemistry system comprising a reaction well block and a daughter plate;

9 FIG. 23B shows a cross-sectional view of the chip-based combinatorial chemistry
system of FIG. 23A taken along line 23B-23B;

10 FIGS. 24A and 24B shows a real Taylor cone emanating from an integrated silicon
11 chip-based nozzle;

12 FIGS. 24C and 24D are perspective and side cross-sectional views, respectively, of
the electrospray device and mass spectrometry system of FIGS. 24A and 24B;

13 FIG. 24E shows a mass spectrum of 1 $\mu\text{g/mL}$ PPG425 in 50% water, 50% methanol
14 containing 0.1% formic acid, 0.1% acetonitrile and 2 mM ammonium acetate, collected at
a flow rate of 333 nL/min;

15 FIG. 25A shows an exploded perspective view of a liquid chromatography device
16 for homogeneous integration with the electrospray device of the present invention;

17 FIG. 25B shows a cross-sectional view of the liquid chromatography device of FIG.
25A taken along line 25B-25B;

18 FIG. 26 shows a plan view of a liquid chromatography device having an exit orifice
19 forming an off-chip interconnection with an off-chip device;

20 FIG. 27 shows a plan view of a liquid chromatography device having an exit orifice
forming an on-chip interconnection with another on-chip device;

21 FIGS. 28-29 show cross-sectional views of liquid chromatography devices having
22 alternative configurations;

23 FIGS. 30-35 show plan views of liquid chromatography devices having alternative
configurations;

24 FIGS. 36A-46C show an example of a fabrication sequence of the liquid
25 chromatography device;

26

1 FIG. 47 shows a cross-sectional view of a system comprising a liquid
2 chromatography device homogenously integrated with an electrospray device;

3 FIG. 48 shows a plan view of the system of FIG. 47; and

4 FIG. 49 shows a detailed view of the nozzles of the system of FIG. 47.

5 DETAILED DESCRIPTION OF THE INVENTION

6 An aspect of the present invention provides a silicon microchip-based electrospray
7 device for producing electrospray ionization of a liquid sample. The electrospray device may
8 be interfaced downstream to an atmospheric pressure ionization mass spectrometer (API-
9 MS) for analysis of the electrosprayed fluid. Another aspect of the invention is an integrated
10 miniaturized liquid phase separation device, which may have, for example, glass, plastic or
11 silicon substrates integral with the electrospray device. The descriptions that follow present
12 the invention in the context of a liquid chromatograph separation device. However, it will
13 be readily recognized that equivalent devices can be made that utilize other microchip-based
14 separation devices. The following description is presented to enable any person skilled in the
15 art to make and use the invention. Descriptions of specific applications are provided only as
16 examples. Various modifications to the preferred embodiment will be readily apparent to
17 those skilled in the art, and the general principles defined herein may be applied to other
18 embodiments and applications without departing from the spirit and scope of the invention.
19 Thus, the present invention is not intended to be limited to the embodiments shown, but is
20 to be accorded the widest scope consistent with the principles and features disclosed herein.

21 **ELECTROSPRAY DEVICE**

22 FIGS. 2-4 show, respectively, a perspective view, a plan view and a crosssectional
23 view of an electrospray device **100** of the present invention. The electrospray apparatus of
24 the present invention generally comprises a silicon substrate or microchip or wafer **102**
25 defining a channel **104** through substrate **102** between an entrance orifice **106** on an injection
26 surface **108** and a nozzle **110** on an ejection surface **112**. The channel may have any suitable
cross-sectional shape such as circular or rectangular. The nozzle **110** has an inner and an
outer diameter and is defined by a recessed region **114**. The region **114** is recessed from the
ejection surface **112**, extends outwardly from the nozzle **110** and may be annular. The tip
of the nozzle **110** does not extend beyond and is preferably coplanar or level with the ejection
surface **112** to thereby protect the nozzle **110** from accidental breakage.

Preferably, the injection surface **108** is opposite the ejection surface **112**. However,
although not shown, the injection surface may be adjacent to the ejection surface such that

1 the channel extending between the entrance orifice and the nozzle makes a turn within the
2 device. In such a configuration, the electrospray device would comprise two substrates
3 bonded together. The first substrate may define a through-substrate channel extending
4 between a bonding surface and the ejection surface, opposite the bonding surface. The first
5 substrate may further define an open channel recessed from the bonding surface extending
6 from an orifice of the through-substrate channel and the injection surface such that the
7 bonding surface of the second substrate encloses the open channel upon bonding of the first
8 and second substrates. Alternatively, the second substrate may define an open channel
9 recessed from the bonding surface such that the bonding surface of the first substrate
10 encloses the open channel upon bonding of the first and second substrates. In yet another
11 variation, the first substrate may further define a second through-substrate channel while the
12 open channel extends between the two through-substrate channels. Thus, the injection
13 surface is the same surface as the ejection surface.

14 A grid-plane region 116 of the ejection surface 112 is exterior to the nozzle 110 and
15 to the recessed region 114 and may provide a surface on which a layer of conductive material
16 119, including a conductive electrode 120, may be formed for the application of an electric
17 potential to the substrate 102 to modify the electric field pattern between the ejection surface
18 112, including the nozzle tip 110, and the extracting electrode 54. Alternatively, the
19 conductive electrode may be provided on the injection surface 108 (not shown).

20 The electrospray device 100 further comprises a layer of silicon dioxide 118 over
21 the surfaces of the substrate 102 through which the electrode 120 is in contact with the
22 substrate 102 either on the ejection surface 112 or on the injection surface 108. The silicon
23 dioxide 118 formed on the walls of the channel 104 electrically isolates a fluid therein from
24 the silicon substrate 102 and thus allows for the independent application and sustenance of
25 different electrical potentials to the fluid in the channel 104 and to the silicon substrate 102.
26 The ability to independently vary the fluid and substrate potentials allows the optimization
of the electrospray through modification of the electric field line pattern, as described below.
Alternatively, the substrate 102 can be controlled to the same electrical potential as the fluid
when appropriate for a given application.

As shown in FIG. 5, to generate an electrospray, fluid may be delivered to the
entrance orifice 106 of the electrospray device 100 by, for example, a capillary 52 or
micropipette. The fluid is subjected to a potential voltage V_{fluid} via a wire (not shown)
positioned in the capillary 52 or in the channel 104 or via an electrode (not shown) provided
on the injection surface 108 and isolated from the surrounding surface region and the

1 substrate 102 . A potential voltage $V_{\text{substrate}}$ may also be applied to the electrode 120 on the
2 grid-plane 116, the magnitude of which is preferably adjustable for optimization of the
3 electrospray characteristics. The fluid flows through the channel 104 and exits or is ejected
4 from the nozzle 110 in the form of very fine, highly charged fluidic droplets 58. The
5 electrode 54 may be held at a potential voltage V_{extract} such that the electrospray is drawn
6 toward the extracting electrode 54 under the influence of an electric field. As it is the
7 relative electric potentials which affect the electric field, the potential voltages of the fluid,
8 the substrate and the extracting electrode may be easily adjusted and modified to achieve the
9 desired electric field. Generally, the magnitude of the electric field should not exceed the
10 dielectric breakdown strength of the surrounding medium, typically air.

11 In one embodiment, the nozzle 110 may be placed up to 10 mm from the sampling
12 orifice of an API mass spectrometer serving as the extracting electrode 54. A potential
13 voltage V_{fluid} ranging from approximately 500-1000 V, such as 700 V, is applied to the fluid.
14 The potential voltage of the fluid V_{fluid} may be up to 500 V/ μm of silicon dioxide on the
15 surface of the substrate 102 and may depend on the surface tension of the fluid being sprayed
16 and the geometry of the nozzle 110. A potential voltage of the substrate $V_{\text{substrate}}$ of
17 approximately less than half of the fluid potential voltage V_{fluid} , or 0-350 V, is applied to the
18 electrode on the grid-plane 116 to enhance the electric field strength at the tip of the nozzle
19 110. The extracting electrode 54 may be held at or near ground potential V_{extract} (0 V). Thus,
20 a nanoelectrospray of a fluid introduced to the electrospray device 100 at flow rates less than
21 1,000 nL/min is drawn toward the extracting electrode 54 under the influence of the electric
22 field.

23 The nozzle 110 provides the physical asperity for concentrating the electric field
24 lines emanating from the nozzle 110 in order to achieve efficient electrospray. The nozzle
25 110 also forms a continuation of and serves as an exit orifice of the through-substrate channel
26 104. Furthermore, the recessed region 114 serves to physically isolate the nozzle 110 from
the grid-plane region 116 of the ejection surface 112 to thereby promote the concentration
of electric field lines and to provide electrical isolation between the nozzle 110 and the grid-
plane region 116. The present invention allows the optimization of the electric field lines
emanating from the nozzle 110 through independent control of the potential voltage V_{fluid}
of the fluid and nozzle 110 and the potential voltage $V_{\text{substrate}}$ of the electrode on the grid-plane
116 of the ejection surface 112.

In addition to the electrode 120, one or more additional conductive electrodes may
be provided on the silicon dioxide layer 118 on the ejection surface 112 of the substrate 102.

FIGS. 6 and 7 show, respectively, a plan view and a cross-sectional view of an example of an electrospray device **100'** wherein the conductive layer **119** defines three additional electrodes **122, 124, 126** on the ejection surface **112** of the substrate **102**. Because the silicon dioxide layer **118** on the ejection surface **112** electrically isolates the silicon substrate **102** from the additional electrodes **122, 124, 126** on the ejection surface **112** and because the additional electrodes **122, 124, 126** are physically separated from each other, the electrical potential applied to each of the additional electrodes **122, 124, 126** can be controlled independently from each other, from the substrate **102** and from the fluid. Thus, additional electrodes **122, 124, 126** may be utilized to further modify the electric field line pattern to effect, for example, a steering and/or shaping of the electrospray. Although shown to be of similar sizes and shapes, electrode **120** and additional electrodes **122, 124, 126** may be of any same or different suitable shapes and sizes.

To further control and optimize the electrospray, a feedback control circuit **130** as shown in FIG. 8 may also be provided with the electrospray device **100**. The feedback circuit **130** includes an optimal spray attribute set point **132**, a comparator and voltage control **134** and one or more spray attribute sensors **136**. The optimal spray attribute set point **132** is set by an operator or at a determined or default value. The one or more spray attribute sensors **136** detect one or more desired attributes of the electrospray from the electrospray device **100**, such as the electrospray ion current and/or the spatial concentration of the spray pattern. The spray attribute sensor **136** sends signals indicating the value of the desired attribute of the electrospray to the comparator and voltage control **134** which compares the indicated value of the desired attribute with the optimal spray attribute set point **132**. The comparator and voltage control **134** then applies potential voltages V_{fluid} , $V_{\text{substrate}}$ to the fluid and the silicon substrate **102**, respectively, which may be independently varied to optimize the desired electrospray attribute. Although not shown, the comparator and voltage control **134** may apply independently controlled additional potential voltages to each of one or more additional conductive electrodes.

The feedback circuit **130** may be interfaced with the electrospray device **100** in any suitable fashion. For example, the feedback circuit **130** may be fabricated as an integrated circuit on the electrospray device **100**, as a separate integrated circuit with electrical connection to the electrospray device **100**, or as discrete components residing on a common substrate electrically connected to the substrate of the electrospray device.

Dimensions of the electrospray device **100** can be determined according to various factors such as the specific application, the layout design as well as the upstream and/or

1 downstream device to which the electrospray device **100** is interfaced or integrated. Further,
2 the dimensions of the channel and nozzle may be optimized for the desired flow rate of the
3 fluid sample. The use of reactive-ion etching techniques allows for the reproducible and cost
4 effective production of small diameter nozzles, for example, a 2 μm inner diameter and 5 μm
outer diameter.

5 In one currently preferred embodiment, the silicon substrate **102** of the electrospray
6 device **100** is approximately 250-600 μm in thickness and the cross-sectional area of the
7 channel **104** is less than approximately 50,000 μm^2 . Where the channel **104** has a circular
8 cross-sectional shape, the channel **104** and the nozzle **110** have an inner diameter of up to
9 250 μm , more preferably up to 145 μm ; the nozzle **110** has an outer diameter of up to 255
10 μm , more preferably up to 150 μm ; and nozzle **110** has a height of (and the recessed portion
11 **114** has a depth of) up to 500 μm . The recessed portion **114** preferably extends up to 1000
12 μm outwardly from the nozzle **110**. The silicon dioxide layer **118** has a thickness of
approximately 1-4 μm , preferably 1-2 μm .

13 **ELECTROSPRAY DEVICE FABRICATION PROCEDURE**

14 The fabrication of the electrospray device **100** will now be explained with reference
15 to FIGS. 9-20B. The electrospray device **100** is preferably fabricated as a monolithic silicon
16 integrated circuit utilizing established, well-controlled thin-film silicon processing
17 techniques such as thermal oxidation, photolithography, reactivation etching (RIE), ion
18 implantation, and metal deposition. Fabrication using such silicon processing techniques
19 facilitates massively parallel processing of similar devices, is time- and cost-efficient, allows
20 for tighter control of critical dimensions, is easily reproducible, and results in a wholly
integral device, thereby eliminating any assembly requirements. Further, the fabrication
sequence may be easily extended to create physical aspects or features on the injection
surface and/or ejection surface of the electrospray device to facilitate interfacing and
connection to a fluid delivery system or to facilitate integration with a fluid delivery sub-
system to create a single integrated system.

21 **Injection surface processing: entrance to through-wafer channel**

22 FIGS. 9A-11 illustrate the processing steps for the injection side of the substrate in
23 fabricating the electrospray device **100** of the present invention. Referring to the plan and
24 cross-sectional views, respectively, of FIGS. 9A and 9B, a double-side polished silicon wafer
25 substrate **200** is subjected to an elevated temperature in an oxidizing ambient to grow a layer
26 or film of silicon dioxide **202** on the injection side **203** and a layer or film of silicon dioxide
204 on the ejection side **205** of the substrate **200**. Each of the resulting silicon dioxide layers

1 202, 204 has a thickness of approximately 1-2 μm . The silicon dioxide layers 202, 204
2 provide electrical isolation and also serve as masks for subsequent selective etching of
certain areas of the silicon substrate 200.

3 A film of positive-working photoresist 206 is deposited on the silicon dioxide layer
4 202 on the injection side 203 of the substrate 200. An area of the photoresist 206
5 corresponding to the entrance to a through-wafer channel which will be subsequently etched
6 is selectively exposed through a mask by an optical lithographic exposure tool passing short-
wavelength light such as blue or near-ultraviolet at wavelengths of 365, 405, or 436
7 nanometers.

8 As shown in the plan and cross-sectional views, respectively, of FIGS. 10A and 10B,
9 after development of the photoresist 206, the exposed area 208 of the photoresist is removed
and open to the underlying silicon dioxide layer 202 while the unexposed areas remain
10 protected by photoresist 206'. The exposed area 210 of the silicon dioxide layer 202 is then
11 etched by a fluorine-based plasma with a high degree of anisotropy and selectivity to the
protective photoresist 206' until the silicon substrate 200 is reached. The remaining
12 photoresist is removed in an oxygen plasma or in an actively oxidizing chemical bath like
sulfuric acid (H_2SO_4) activated with hydrogen peroxide (H_2O_2).
13

14 As shown in the cross-sectional view of FIG. 11, an injection side portion 212 of the
through channel in the silicon substrate 200 is vertically etched by another fluorine-based
15 etch. An advantage of the fabrication process described herein is that the dimensions of the
through channel, such as the aspect ratio (depth to width), can be reliably and reproducibly
16 limited and controlled. In the case where the etch aspect ratio of the processing equipment
is a limiting factor, it is possible to overcome this limitation by a first etch on one side of a
17 wafer followed by a second etch on a second side of the wafer. For example, a current
silicon etch process is generally limited to an etch aspect ratio of 30:1, such that a channel
18 having a diameter less than approximately 10 μm through a substrate 200 having customary
thickness approximately 250-600 μm would be etched from both surfaces of the substrate
19 200.
20

21 The depth of the channel portion 212 should be at or above a minimum in order to
22 connect with another portion of the through channel etched from the ejection side 205 of the
substrate 200. The desired depth of the recessed region 114 on the ejection side 205
23 determines approximately how far the ejection side portion 220 of the channel 104 is etched.
The remainder of the channel 104, the injection side portion 212, is etched from the injection
24 side. The minimum depth of channel portion 212 is typically 50 μm , although the exact etch
25
26

1 depth above the minimum etch depth does not impact the device performance or yield of the
2 electro spray device.

3 **Ejection surface processing: nozzle and surrounding surface structure**

4 FIGS. 12-20B illustrate the processing steps for the ejection side 205 of the substrate
5 200 in fabricating the electro spray device 100 of the present invention. As shown in the
6 cross-sectional view in FIG. 12, a film of positive-working photoresist 214 is deposited on
7 the silicon dioxide layer 204 on the ejection side 205 of the substrate 200. Patterns on the
8 ejection side 205 are aligned to those previously formed on the injection side 203 of the
9 substrate 200. Because silicon and its oxide are inherently relatively transparent to light in
10 the infrared wavelength range of the spectrum, i.e. approximately 70-1000 nanometers, the
11 extant pattern on the injection side 203 can be distinguished with sufficient clarity by
12 illuminating the substrate 200 from the patterned injection side 203 with infrared light. Thus,
13 the mask for the ejection side 205 can be aligned within required tolerances.

14 After alignment, certain areas of the photoresist 214 corresponding to the nozzle and
15 the recessed region are selectively exposed through an ejection side mask by an optical
16 lithographic exposure tool passing short-wavelength light, such as blue or near-ultraviolet
17 at wavelengths of 365, 405, or 436 nanometers. As shown in the plan and cross-sectional
18 views, respectively, of FIGS. 13A and 13B, the photoresist 214 is then developed to remove
19 the exposed areas of the photo resist such that the nozzle area 216 and recessed region area
20 218 are open to the underlying silicon dioxide layer 204 while the unexposed areas remain
21 protected by photoresist 214'. The exposed areas 216, 218 of the silicon dioxide layer 204
22 are then etched by a fluorine-based plasma with a high degree of anisotropy and selectivity
23 to the protective photoresist 214' until the silicon substrate 200 is reached.

24 As shown in the cross-sectional view of FIG. 14, the remaining photoresist 214'
25 provides additional masking during a subsequent fluorine based silicon etch to vertically etch
26 certain patterns into the ejection side 205 of the silicon substrate 200. The remaining
photoresist 214' is then removed in an oxygen plasma or in an actively oxidizing chemical
bath like sulfuric acid (H_2SO_4) activated with hydrogen peroxide (H_2O_2).

The fluorine-based etch creates a channel 104 through the silicon substrate 200 by
forming an ejection side portion 220 of the channel 104. The fluorine based etch also creates
an ejection nozzle 110, a recessed region 114 exterior to the nozzle 110 and a grid-plane
region 116 exterior to the nozzle 110 and to the recessed region 114. The grid-plane region
116 is preferably co-planar with the tip of the nozzle 110 so as to physically protect the
nozzle 110 from casual abrasion, stress fracture in handling and/or accidental breakage. The

1 grid-plane region 116 also serves as a platform on which one or more conductive electrodes
2 may be provided.

3 The fabrication sequence confers superior mechanical stability to the fabricated
4 electrospray device by etching the features of the electrospray device from a monocrystalline
5 silicon substrate without any need for assembly. The fabrication sequence allows for the
6 control of the nozzle height by adjusting the relative amounts of injection side and ejection
7 side silicon etching. Further, the lateral extent and shape of the recessed region 114 can be
8 controlled independently of its depth, which affects the nozzle height and which is
9 determined by the extent of the etch on the ejection side of the substrate. Control of the
10 lateral extent and shape of the recessed region 114 provides the ability to modify and control
11 the electric field pattern between the electrospray device 100 and an extracting electrode.

12 **Oxidation for electrical isolation**

13 As shown in the cross-sectional view of FIG. 15, a layer of silicon dioxide 221 is
14 grown on all silicon surfaces of the substrate 200 by subjecting the silicon substrate 200 to
15 elevated temperature in an oxidizing ambient. For example, the oxidizing ambient may be
16 an ultra-pure steam produced by oxidation of hydrogen for a silicon dioxide thickness greater
17 than approximately several hundred nanometers or pure oxygen for a silicon dioxide
18 thickness of approximately several hundred nanometers or less. The layer of silicon dioxide
19 221 over all silicon surfaces of the substrate 200 electrically isolates a fluid in the channel
20 from the silicon substrate 200 and permits the application and sustenance of different
21 electrical potentials to the fluid in the channel 104 and to the silicon substrate 200.

22 All silicon surfaces are oxidized to form silicon dioxide with a thickness that is
23 controllable through choice of temperature and time of oxidation. The final thickness of the
24 silicon dioxide can be selected to provide the desired degree of electrical isolation in the
25 device, where a thicker layer of silicon dioxide provides a greater resistance to electrical
26 breakdown.

27 **Metallization for electric field control**

28 FIGS. 16-20B illustrate the formation of a single conductive electrode electrically
29 connected to the substrate 200 on the ejection side 205 of the substrate 200. As shown in the
30 cross-sectional view of FIG. 16, a film of positive-working photoresist 222 is deposited over
31 the silicon dioxide layer on the ejection side 205 of the substrate 200. An area of the
32 photoresist 222 corresponding to the electrical contact area between the electrode and the
33 substrate 200 is selectively exposed through another mask by an optical lithographic

1 exposure tool passing short-wavelength light, such as blue or near-ultraviolet at wavelengths
2 of 365, 405, or 436 nanometers.

3 The photoresist **222** is then developed to remove the exposed area **224** of the
4 photoresist such that the electrical contact area between the electrode and the substrate **200**
5 is open to the underlying silicon dioxide layer **204** while the unexposed areas remain
6 protected by photoresist **222'**. The exposed area **224** of the silicon dioxide layer **204** is then
7 etched by a fluorine-based plasma with a high degree of anisotropy and selectivity to the
8 protective photoresist **222'** until the silicon substrate **200** is reached, as shown in the cross-
9 sectional view of FIG. 17.

10 Referring now to the cross-sectional view of FIG. 18, the remaining photoresist is
11 then removed in an oxygen plasma or in an actively oxidizing chemical bath like sulfuric
12 acid (H_2SO_4) activated with hydrogen peroxide (H_2O_2). Utilizing the patterned ejection side
13 silicon dioxide layer **204** as a mask, a high-dose implantation is made to form an implanted
14 region **225** to ensure a low-resistance electrical connection between the electrode and the
15 substrate **200**. A conductive film **226** such as aluminum may be uniformly deposited on the
16 ejection side **205** of the substrate **200** by thermal or election-beam evaporation to form an
17 electrode **120**. The thickness of the conductive film **226** is preferably approximately 3000
18 Å, although shown having a larger thickness for clarity.

19 The conductive film **226** may be created by any method which does not produce a
20 continuous film of the conductive material on the side walls of the ejection nozzle **110**. Such
21 a continuous film would electrically connect the fluid in the channel **104** and the substrate
22 **200** so as to prevent the independent control of their respective electrical potentials. For
23 example, the conductive film may be deposited by thermal or electron-beam evaporation of
24 the conductive material, resulting in line-of-sight deposition on presented surfaces. Orienting
25 the substrate **200** such that the side walls of the ejection nozzle **110** are out of the line-of-
26 sight of the evaporation source ensures that no conductive material is deposited as a
continuous film on the side walls of the ejection nozzle **110**. Sputtering of conductive
material in a plasma is an example of a deposition technique which would result in
deposition of conductive material on all surfaces and thus is undesirable.

One or more additional conductive electrodes may be easily formed on the ejection
side **205** of the substrate **200**, as described above with reference to FIGS. 6 and 7. As shown
in the cross-sectional view of FIG. 19, a film of positive-working photoresist **228** is
deposited over the conductive film **226** on the ejection side **205** of the substrate **200**. Certain
areas of the photoresist **228** corresponding to the physical spaces between the electrodes are

1 selectively exposed through another mask by an optical lithographic exposure tool passing
2 short-wavelength light, such as blue or near-ultraviolet at wavelengths of 365, 405, or 436
nanometers.

3 Referring now to the plan and cross-sectional views of FIGS. 20A and 20B, the
4 photoresist **228** is developed to remove the exposed areas **230** of the photoresist such that the
5 exposed areas are open to the underlying conductive film **226** while the unexposed areas
6 remain protected by photoresist **228'**. The exposed areas **230** of the conductive film **226** are
7 then etched using either a wet chemical etch or a reactive-ion etch, as appropriate for the
8 particular conductive material. The etch is either selective to the underlying silicon dioxide
layer **204** or the etch must be terminated on the basis of etch rate and time of etch. Finally,
the remaining photoresist is then removed in an oxygen plasma.

9 The etching of the conductive film **226** to the underlying silicon dioxide layer **204**
10 results in physically and electrically separate islands of conductive material or electrodes.
11 As described above, these electrodes can be controlled independently from the silicon
12 substrate or channel fluid because they are electrically isolated from the substrate by the
13 silicon dioxide and from each other by physical separation. They can be used to further
14 modify the electric field line pattern and thereby effect a steering and/or shaping of the
electrosprayed fluid. This step completes the processing and fabrication sequence for the
electrospray device **100**.

15 As described above, the conductive electrode for application of an electrical
16 potential to the substrate of the electrospray device may be provided on the injection surface
17 rather than the ejection surface. The fabrication sequence is similar to that for the conductive
18 electrode provided on the ejection side **205** of the substrate **200**. FIGS. 20C-20G illustrate
19 the formation of a single conductive electrode electrically connected to the substrate **200** on
the injection side **203** of the substrate **200**.

20 As shown in the cross-sectional view of FIG. 20C, a film of positive-working
21 photoresist **232** is deposited over the silicon dioxide layer on the injection side **203** of the
22 substrate **200**. An area of the photoresist **232** corresponding to the electrical contact area
23 between the electrode and the substrate **200** is selectively exposed through another mask by
24 an optical lithographic exposure tool passing shortwavelength light, such as blue or near-
ultraviolet at wavelengths of 365, 405, or 436 nanometers.

25 The photoresist **232** is then developed to remove the exposed area **234** of the
26 photoresist such that the electrical contact area between the electrode and the substrate **200**
is open to the underlying Silicon dioxide layer **202** while the unexposed areas remain

1 protected by photoresist **232'**. The exposed area **234** of the silicon dioxide layer **202** is then
2 etched by a fluorine-based plasma with a high degree of anisotropy and selectivity to the
3 protective photoresist **232'** until the silicon substrate **200** is reached, as shown in the cross-
sectional view of FIG. 20D.

4 Referring now to the cross-sectional view of FIG. 20E, the remaining photoresist is
5 then removed in an oxygen plasma or in an actively oxidizing chemical bath like sulfuric
6 acid (H_2SO_4) activated with hydrogen peroxide (H_2O_2). Utilizing the patterned injection side
7 silicon dioxide layer **202** as a mask, a high-dose implantation is made to form an implanted
8 region **236** to ensure a low-resistance electrical connection between the electrode and the
9 substrate **200**. A conductive film **238** such as aluminum may be uniformly deposited on the
injection side **203** of the substrate **200** by thermal or electron beam evaporation to form an
electrode **120'**.

10 In contrast to the formation of the conductive electrode on the ejection surface of the
11 electrospray device, sputtering, in addition to thermal or electron-beam evaporation, may be
12 utilized to form the conductive electrode on the injection surface. Because the nozzle is on
13 the ejection rather than the injection side of the substrate, sputtering may be utilized to form
14 the electrode on the injection side as the injection side electrode layer does not extend to the
nozzle to create a physically continuous and thus electrically conductive path with the nozzle.

15 With the formation of the electrode on the injection surface of the electrospray
16 device, sputtering may be preferred over evaporation because of its greater ability to produce
17 conformal coatings on the sidewalls of the exposed area **234** etched through the silicon
dioxide layer **202** to the substrate **200** to ensure electrical continuity and reliable electrical
contact to the substrate **200**.

18 For certain applications, it may be necessary to ensure electrical isolation between
19 the substrate **200** and the fluid in the electrospray device by removing the conductive film
20 from the region of the surface adjacent to the entrance orifice **106** on the injection side **203**.
21 The extent of the conductive film **238** which should be removed is irrespective of etching
22 method and may be determined by the specific method utilized in creating the interface
23 between the upstream fluid delivery system/sub-system and the injection side of the
electrospray device. For example, a diameter of between approximately 0.2-2 mm of the
conductive film **238** may be removed from the region surrounding the entrance orifice **106**.

24 As shown in the cross-sectional view of FIG. 20F, another film of positive-working
25 photoresist **240** is deposited over the conductive film **238** on the injection side **203** of the
26 substrate **200**. An area of the photoresist **240** corresponding to the region adjacent to the

1 entrance orifice **106** on the injection side **203** is selectively exposed through another mask
2 by an optical lithographic exposure tool passing short-wavelength light, such as blue or near-
ultraviolet at wavelengths of 365, 405, or 436 nanometers.

3 The photoresist **240** is then developed to remove the exposed area **242** of the
4 photoresist such that the region adjacent to the entrance orifice **106** on the injection side **203**
5 is open to the underlying conductive film **238** while the unexposed areas remain protected
6 by photoresist **240'**. The exposed area **242** of the conductive film **238** is then etched by, for
7 example, a chlorine-based plasma with a high degree of anisotropy and selectivity to the
8 protective photoresist **240'** until the silicon dioxide layer **203** is reached, as shown in the
cross-sectional view of FIG. 20G.

9 The specific technique for etching the conductive film **238** may be determined by
10 the specific conductive material deposited. For example, aluminum may be etched either in
11 a wet chemical bath using standard aluminum etchant or in a plasma using reactive-ion
12 etching (RIE) and chlorine-based gas chemistry. Utilization of standard wet aluminum
13 etchant to etch an aluminum film may be preferred as such wet etching may facilitate the
14 removal of any undesired conductive material deposited in the channel **104** via the entrance
orifice **106**. Further, although chlorine-based reactive-ion etching may be utilized, such
etching may lead to aluminum corrosion if removal of the photoresist is delayed.

15 Forming the electrode on the injection surface for application of an electric potential
16 to the substrate of the electrospray device may provide several advantages. For example,
17 because the ability to uniformly coat photoresist on a surface is limited by nonplanar surface
18 topology, coating photoresist on the much flatter injection side results in a more uniform and
19 continuous photoresist film than coating photoresist on the ejection side. The uniformity and
20 continuity of the photoresist film directly and positively impact the reliability and yield, at
least in part because failure of photoresist coverage would allow subsequent etching of
silicon dioxide in undesired locations during the etching of exposed areas **224, 234**.

21 Another advantage of forming the electrode on the injection surface is the greater
22 flexibility and reliability in the conductive material deposition step because the interior
23 surfaces of the nozzle are not coated by the conductive material deposited onto the injection
24 surface rather than onto the ejection surface of the electrospray device. As a result,
sputtering may be utilized as a deposition technique to ensure conformal coating of the
conductive material and electrical continuity from the surface to the substrate contact.
25 Further, the provision of the electrode on the injection surface does not preclude the
26 deposition and patterning of additional conductive electrodes on the ejection side to further

1 modify the electric field line pattern to effect, for example, a steering and/or shaping of the
2 electrospray, as such additional electrodes do not required electrical contact to the substrate.

3 The ability to form the electrode on the injection surface may also be advantageous
4 in certain applications where physical constraints, such as in packaging, may dictate the need
5 for injection-side rather than ejection-side electrical connection.

6 The above described fabrication sequence for the electrospray device 100 can be
7 easily adapted to and is applicable for the simultaneous fabrication of a single monolithic
8 system comprising multiple electrospray devices including multiple channels and/or multiple
9 ejection nozzles embodied in a single monolithic substrate. Further, the processing steps
10 may be modified to fabricate similar or different electrospray devices merely by, for example,
11 modifying the layout design and/or by changing the polarity of the photomask and utilizing
12 negative-working photoresist rather than utilizing positive-working photoresist.

13 Further, although the fabrication sequence is described in terms of fabricating a
14 single electrospray device, the fabrication sequence facilitates and allows for massively
15 parallel processing of similar devices. The multiple electrospray devices or systems
16 fabricated by massively parallel processing on a single wafer may then be cut or otherwise
17 separated into multiple devices or systems.

18 **INTERFACE OR INTEGRATION OF THE ELECTROSPRAY DEVICE**

19 **Downstream Interface or Integration of the Electrospray Device**

20 The electrospray device 100 may be interfaced or integrated downstream to a
21 sampling device, depending on the particular application. For example. the analyte may be
22 electrosprayed onto a surface to coat that surface or into another device for purposes of
23 conveyance, analysis, and/or synthesis. As described above with reference to FIG. 5, highly
24 charged droplets are formed at atmospheric pressure by the electrospray device 100 from
25 nanoliter-scale volumes of an analyte. The highly charged droplets produce gas-phase ions
26 upon sufficient evaporation of solvent molecules which may be sampled, for example,
through an orifice of an atmospheric pressure ionization mass spectrometer (API-MS) for
analysis of the electrosprayed fluid.

Upstream Interface or Integration of the Electrospray Device

Referring now to FIGS. 21-23, fluid may be delivered to the entrance orifice of the
electrospray device in any suitable manner by upstream interface or integration with one or
more fluid delivery devices, such as piezoelectric pipettes, micropipettes, capillaries and
other types of microdevices. The fluid delivery device may be a separate component to form
a heterogeneous interface with the entrance orifice of the electrospray device. Alternatively,

1 the fluid delivery device may be integrated with the electrospray device to form a
2 homogeneous interface with the entrance orifice of the electrospray device.

3 FIGS. 21A and 21B illustrate examples of fluid delivery devices forming
4 heterogeneous interfaces with the entrance orifice of the electrospray device. Preferably, the
5 heterogeneous interface is a non-contacting interface where the fluid delivery device
6 and the electrospray device are physically separated and do not contact. For example, as
7 shown in the cross-sectional view of FIG. 21A, a piezoelectric pipette 300 is positioned at
8 a distance above the injection surface 108 of the electrospray device 100A. The piezoelectric
9 pipette 300 deposits a flow of microdroplets, each approximately 200 pL in volume, into the
10 channel 104 through the entrance orifice 106A. Preferably, the electrospray device 100A
11 provides an entrance well 302 at the entrance orifice 106A for containing the sample fluid
12 prior to entering the channel 104 particularly when it is desirable to spray a volume of fluid
13 greater than the volume of the through-substrate channel 104 and continual supply of fluid
14 is not feasible such as when using the piezoelectric pipette 300. The entrance well 302
15 preferably has a volume of 0.1 nL to 100 nL. Furthermore, to apply an electric potential to
16 the fluid, an entrance well electrode 304 may be provided on a surface of the entrance well
17 302 parallel to the injection surface 108. Alternatively, a wire (not shown) may be
18 positioned in channel 104 via the entrance orifice 106A. Preferably, some fluid is present
19 in the entrance well 302 to ensure electrical contact between the fluid and the entrance well
20 electrode 304.

21 Alternatively, the heterogeneous interface may be a contacting interface where a
22 fluid delivery device is attached by any suitable method, such as by epoxy bonding, to the
23 electrospray device to form a continuous sealed flow path between the upstream fluid source
24 and the channel of the electrospray device. For example, FIG. 21B shows a cross-sectional
25 view of a capillary 306 prior to attachment to the entrance orifice 106 of the electrospray
26 device 100B. The injection surface 108 of the electrospray device 100B may be adapted to
facilitate attachment of the capillary 306. Such features can be easily designed into the mask
for the injection side of the substrate and can be simultaneously formed with the injection
side portion of the channel during the etching performed on the injection-side.

For example, where the inner diameter of the capillary 306 is greater than that of the
channel 104 and the entrance orifice 106, the electrospray device 100B preferably defines a
region 308 recessed from the injection surface 108 to form a mating collar for mating and
affixing with the capillary 306. Thus, capillary 306 may be positioned and attached in the
recessed region 308 such that the exit orifice 310 portion of the capillary 302 is positioned

1 around the entrance orifice **106**. Further, the electrospray device **100B** may optionally
2 provide an entrance well **312** at the entrance orifice **106B** for containing the sample fluid
3 prior to entering the channel **104**. Although not shown, if the outer diameter of the capillary
4 is less than that of the channel and the entrance orifice, the capillary may be inserted into and
attached to the entrance orifice of the electrospray device.

5 Referring now to the schematic of FIG. 22, rather than a heterogeneous interface,
6 a single integrated system **316** is provided wherein an upstream fluid delivery device **318**
7 forms a homogeneous interface with the entrance orifice (not shown) of an electrospray
8 device **100**. The system **316** allows for the fluid exiting the upstream fluid delivery device
9 **318** to be delivered on-chip to the entrance orifice of the electrospray device **100** in order to
generate an electrospray.

10 The single integrated system **316** provides the advantage of minimizing or
11 eliminating extra fluid volume to reduce the risk of undesired fluid changes, such as by
12 reactions and/or mixing. The single integrated system **316** also provides the advantage of
13 eliminating the need for unreliable handling and attachment of components at the
microscopic level and of minimizing or eliminating fluid leakage by containing the fluid
within one integrated system.

14 The upstream fluid delivery device **318** may be a monolithic integrated circuit
15 having an exit orifice through which a fluid sample can pass directly or indirectly to the
16 entrance orifice of the electrospray device **100**. The upstream fluid delivery device **318** may
17 be a silicon microchip-based liquid separation device capable of, for example, capillary
18 electrophoresis, capillary electrochromatography, affinity chromatography, liquid
19 chromatography (LC) or any other condensed-phase separation methods. Further, the
20 upstream fluid delivery device **318** may be a silicon, glass, plastic and/or polymer based
device such that the electrospray device **100** may be chip-to-chip or wafer-to-wafer bonded
thereto by any suitable method. An example of a monolithic liquid chromatography device
for utilization in, for example, the single integrated system **316**, is described below.

21 **Electrospray Device for Sample Transfer of Combinatorial Chemistry**
22 **Libraries Synthesized in Microdevices**

23 The electrospray device may also serve to reproducibly distribute and deposit a
24 sample from a mother plate to daughter plate(s) by nanoelectrospray deposition. Electrospray
25 device(s) may be etched into a microdevice capable of synthesizing combinatorial chemical
26 libraries. At the desired time, the nozzle may spray a desired amount of the sample from the
mother plate to the daughter plate(s). Control of the nozzle dimensions, applied voltages,

1 and time of spraying may provide a precise and reproducible method of sample deposition
2 from an array of nozzles, such as the generation of sample plates for molecular weight
3 determinations by matrix-assisted laser desorption/ionization time-of-flight mass
4 spectrometry (MALDI-TOFMS). The capability of transferring analytes from a mother plate
5 to daughter plates may also be utilized to make other daughter plates for other types of
assays, such as proteomic screening.

6 FIGS. 23A and 23B show; respectively, an exploded perspective view and a cross-
7 sectional view along line 23B-23B, of a chip-based combinatorial chemistry system 320
8 comprising a reaction well block or titer plate 322 and a receiving or daughter plate 324. The
9 reaction well block 322 defines an array of reservoirs 326 for containing the reaction
10 products from a combinatorially synthesized compound. The reaction well block 322 further
11 defines channels 328, nozzles 330 and recessed portions 332 such that the fluid in each
12 reservoir 326 may flow through a corresponding channel 328 and exit through a
13 corresponding nozzle 330 in the form of an electrospray. The reaction well block 322 may
14 define any number of reservoir(s) in any desirable configuration, each reservoir being of a
suitable dimension and shape. The volume of a reservoir 326 may range from a few
nanoliters up to several microliters and more preferably ranges between approximately 200
nL to 1 μ L.

15 The reaction well block 322 may serve as a mother plate to interface to a microchip-
16 based chemical synthesis apparatus such that the electrospray function of the reaction well
17 block 322 may be utilized to reproducibly distribute discreet quantities of the product
18 solutions to a receiving or daughter plate 324. The daughter plate 324 defines receiving
19 wells 334 which correspond to each of the reservoirs 326. The distributed product solutions
in the daughter plate 324 may then be utilized to screen the combinatorial chemical library
against biological targets.

20 **Illustration of an Electrospray Device Generating an Electrospray Spray**

21 FIGS. 24A and 24B show color images of a real Taylor cone emanating from an
22 integrated silicon chip-based nozzle. FIGS. 24C and 24D are perspective and side cross-
23 sectional views, respectively, of the electrospray device and mass spectrometer system shown
24 in FIGS. 24A and 24B. FIGS. 24A shows a chip-integrated electrospray device comprising
a nozzle and a recessed portion or annulus, and a Taylor cone, liquid jet and plume of highly-
charged electrosprayed droplets of methanol containing 10 μ g/mL polypropylene glycol 425
(PPG425) containing 0.2% formic acid. FIG. 24B shows an ion-sampling orifice of a mass
spectrometer in addition to the electrospray device.

1 The electrospray device 100 is interfaced upstream with a pipette 52'. As shown in
2 the upper right corner of each of FIGS. 24A and 24B and in FIGS. 24C and 24D, the tip of
3 the pipette 52' is press-sealed to the injection side of the electrospray device 100. The
4 electrospray device 100 has a 10 μm diameter entrance orifice on the injection side, a 30 μm
5 inner diameter and a 60 μm outer diameter nozzle, a 15 μm nozzle wall thickness and a 150
6 μm nozzle depth. The recessed portion or the annulus extends 300 μm from the outer
7 diameter of the nozzle. The voltage applied to the fluid V_{fluid} introduced to the electrospray
8 device and thus the nozzle voltage is 900 V. The voltage applied to the substrate $V_{\text{substrate}}$ and
9 thus the electrospray device is 0 V. The voltage applied to the mass spectrometer which also
10 serves as an extracting electrode V_{extract} is approximately 40 V. The liquid sample was
11 pumped using a syringe pump at a flow of 333 nL/min through the pipette tip pressed-sealed
12 against the injection side of the electrospray device. The nozzle is approximately 5 mm from
13 the ion-sampling orifice 62 of the mass spectrometer 60. The ion-sampling orifice 62 of the
14 mass spectrometer 60 generally defines the acceptance region of the mass spectrometer 60.
15 The mass spectrometer for acquiring the data was the LCT Time-Of-Flight mass
16 spectrometer of Micromass, Inc.

17 FIG. 24E shows a mass spectrum of 1 $\mu\text{g/mL}$ PPG425 in 50% water, 50% methanol
18 containing 0.1% formic acid, 0.1% acetonitrile and 2 mM ammonium acetate. The data were
19 collected at a flow rate of 333 nL/min.

20 LIQUID CHROMATOGRAPHY DEVICE

21 In another aspect of the invention shown in the exploded perspective and cross-
22 sectional views of FIGS. 25A and 25B, respectively, a silicon-based liquid chromatography
23 device 400 generally comprises a silicon substrate or microchip 402 defining an introduction
24 channel 404 through the substrate 402 extending between an entrance orifice 406 on a first
25 surface 408 and a fluid reservoir 410, a separation channel 412 extending between the
26 reservoir 410 and an exit orifice 414, a plurality of separation posts 416 along the separation
channel 412, and a cover 420 to provide an enclosure surface adjacent the cover 420 for the
reservoir 410 and the separation channel 412 adjacent the cover 420.

The plurality of separation posts 416 extends from a side wall of the separation
channel 412 in a direction perpendicular to the fluid flow through the separation channel 412.
Preferably, one of the ends of each separation post 416 does not extend beyond and is
preferably coplanar or level with the second surface 417. The separation channel 412 is
functionally similar to the liquid chromatography column in that component separation
occurs in the separation channel 412 where the plurality of separation posts 416 perform the

Depending on the specific application, the substrate **402** may provide a surface on which one or more conductive electrodes in electrical contact with the fluid in the device **400** may be formed. For example, a reservoir electrode **426** and/or an exit electrode **428** may be provided on the second surface **417** of the substrate **402** such that a corresponding electrode would be in electrical contact with fluid in the reservoir **410** and near the exit orifice **414**, respectively. A filling electrode **430** may also be provided on the second surface **417** of the substrate **402** such that it would be in electrical contact with fluid in the unpopulated portion **432** of the separation channel **412** between the reservoir **410** and the first occurrence of separation posts **416**. The shape, size and location along the fluidic flow path of each electrode on the substrate **402** may be determined by design considerations such as the distance between adjacent electrodes. Further, any or all of the electrodes may be alternatively or additionally formed on the bonding surface **425** of the cover **420**. For example, the filling electrode **430** may be alternatively positioned such that it would be in

1 electrical contact with fluid in the separation channel **412** adjacent the reservoir **410**.
2 Further, additional electrodes may be provided, for example, to create an arbitrary electrical
3 potential distribution along the fluidic flow path.

4 Providing two or more of the reservoir, filling and exit electrodes along with
5 electrical isolation of the fluid sample in the device **400** from the substrate **402** and the
6 substrate of the cover **420** allows for the application and sustenance of different (or same)
7 electric potentials at two or more different locations along the fluidic path. The difference
8 in electric potentials at two or more different locations along the fluidic path causes fluidic
9 motion to occur between the two or more locations. Thus, these electrodes may facilitate the
10 filling of the reservoir **410** and/or the driving of the fluid through the separation channel **412**.

11 Further, through appropriate layout design and fabrication processes, the substrate
12 **402** and/or the cover **420** may also provide additional functionalities such as pre-conditioning
13 of the fluid prior to delivery into the reservoir **410**, and/or conveying, analyzing, and/or
14 otherwise treating fluidic samples exiting from the separation channel **412**. The cover **420**
15 may provide such additional functionality on either or both surfaces and/or the bulk of the
16 cover **420**.

17 The cover **420** may comprise a substrate **418** comprising silicon or any other suitable
18 material, such as glass, plastics and/or polymers. The specific material for the cover **420** may
19 depend upon, for example, whether direct observation of a fluoresced fluid is desired such
20 that glass may be more desirable and/or the consideration of the ease of fabrication of the
21 cover **420** by utilizing similar processing techniques as for the substrate **402** such that silicon
22 may be more desirable. The cover **420** may be bonded or otherwise affixed to form a
23 hermetic seal between the substrate **402** and the cover **420** in order to ensure the appropriate
24 level of fluid containment and isolation. For example, several methods of bonding silicon
25 to silicon or glass to silicon are known in the art, including anodic bonding, sodium silicate
26 bonding, eutectic bonding, and fusion bonding. The specific hermetic bonding method may
depend on various factors such as the physical form of the surfaces of the substrate **402** and
the cover **420** and/or the application and functionality of the integrated system and/or the
liquid chromatography device **400**.

Dimensions of the liquid chromatography device **400** may be determined according
to various factors such as the specific application, the layout design as well as the device with
which it is to be interfaced or integrated. The surface dimensions, i.e. the dimensions in the
X and Y directions, of the elements of the liquid chromatography device **400** may be
determined by layout design and through the corresponding photomasks used in fabrication.

1 The depth or height, i.e. the dimension in the Z direction, of the elements of the liquid
2 chromatography device 400 may be determined by the etch processes during fabrication, as
3 described below. The depth or height of the elements is independent of the surface
4 dimensions to a first-order approximation although the aspect ratio limitations of the
5 reactive-ion etch places constraints on the etch depth, particularly with the small surface
openings in the channel 412 between the separation posts 416.

6 Further, the size, number, cross-sectional shape, spacing and placement of the
7 separation posts 416 may also be determined by layout design to achieve the desired flow
8 rate and to prevent low-resistance lines of sight within the separation channel 412 to ensure
9 adequate fluid-surface interaction. Each separation post 416 may have the same or different
10 characteristics such as size and/or cross-sectional shape. The cross-sectional shape of the
11 posts may be chosen in layout design to optimize fluid/boundary layer interactions at the post
12 surfaces. The separation posts 416 may be placed in any desired pattern in the separation
13 channel 412, such as periodic, semi-periodic, or random. Close spacing of the separation
14 posts 416 may be desirable for maximization of the surface interactions with the fluid.
15 Similarly, minimizing the cross-sectional area of the separation posts 416 may permit
16 placement of greater number in the separation channel 412. However, the reduction of the
17 cross-sectional area of the separation posts 416 is limited by the resulting reduction in the
18 mechanical stability necessary during processing.

19 Control of the size, number, cross-sectional shape, spacing and placement of the
20 separation posts 416 provides advantages over traditional liquid chromatography as the
21 traditional separation column packing materials have undesired dispersion in size distribution
22 as well as random spacing variations.

23 In one currently preferred embodiment, the substrate 402 of the liquid
24 chromatography device 400 is approximately 250-600 μm in thickness, the separation
25 channel 412 has a depth of approximately 10 μm , the rectangular reservoir 410 is
26 approximately 1000 μm by 1000 μm resulting in a volume of approximately 10 nL. The
depth of the reservoir 410 and the separation channel 412 is limited by the height of the
separation posts 416 which is in turn limited by the maximum etch aspect ratio. The nearest-
neighbor spacing of the separation posts 416 is preferably less than approximately 5 μm .
The dimensions of the reservoir 410 determine the volume of the fluid sample which can be
used for the liquid chromatography separation and, as is evident, through the independent
control of surface dimensions and the depth, the reservoir 410 may be designed to have any
desired volume. Preferably, the diameter of the entrance orifice 406 is 100 μm or less such

1 that the fluid surface tension would be sufficient to maintain the fluid in the reservoir 410
2 to prevent leakage therefrom.

3 The silicon-based liquid chromatography device 400 reduces the size of a typical
4 liquid chromatography device by nearly two orders of magnitude. The dimensional scaling
5 may provide the advantage of significantly reducing the mass of the analyte and/or the
6 volume of the fluid sample required for accurate analysis. Further, by reducing a
macroscopic separation column and its packing materials to a monolithic device, the liquid
chromatography device 400 can be a component of an on-chip integrated system.

7 Further, all features such as the reservoir, the separation channel and the separation
8 posts are recessed from the substrate 402. The portion of the substrate 402 exterior to the
9 reservoir and the separation channel thus serves to physically protect the separation posts
10 from casual abrasion and stress fracture in handling and subsequent bonding of the substrate
11 402 and the cover 420. Because the posts are integral with the substrate, the posts are
12 inherently stable and thus allow for the use of a pressurized system without the risk of
damage to the stationary phase which may otherwise result with the use of conventional
packing materials in conventional high-performance liquid chromatography systems.

13 An upstream fluid delivery system, such as a micropipette, piezoelectric pipette or
14 small capillary, may be press-sealed onto the exterior surface of the liquid chromatography
15 device 400 such that the pipette or capillary is concentric with the entrance orifice 406.
16 Optionally, the liquid chromatography device may provide a collar (not shown) to facilitate
17 the mating and affixing of the fluid delivery device to the liquid chromatography device
similar to the mating collar of the electrospray device as discussed with reference to FIG.
21B.

18 To operate the liquid chromatography device 400, the fluid reservoir 410 may first
19 be filled with a sample fluid by injecting the fluid from a fluid delivery device through the
20 introduction channel 404 via the entrance orifice 406. Any suitable fluid delivery device
21 such as a micropipette, a piezoelectric pipette or a small capillary may be utilized. The
22 volume of the sample fluid injected into the liquid chromatography device 400 may be up
23 to approximately the volume of the reservoir 410 plus a relatively small volume remaining
in the introduction channel 404.

24 The filling of the reservoir 410 may be facilitated by applying an appropriate
25 potential voltage difference between the reservoir electrode 426 and the filling electrode 430,
26 such as approximately 1000 V/cm of introduction channel 404. In particular, a volume of
the fluid is first introduced into the reservoir 410 through the introduction channel 404 via

1 the entrance orifice 406 to coat or prime the surfaces of the reservoir 410 and the
2 introduction channel 404 by capillary action to allow for electrical contact between the fluid
3 and the reservoir and filling electrodes 426, 430. Where the filling electrode 430 is
4 positioned in a portion of the separation channel 412 unpopulated by separation posts 416,
5 the filling electrode 430 also facilitates the filling of the portion of the channel 412 between
6 the reservoir 410 and the filling electrode 430.

7 After filling the reservoir 410 with an appropriate volume of the sample fluid, any
8 suitable method may then be utilized to drive the fluid from the reservoir 410 into the
9 separation channel 412. For example, the fluid may be driven from the filled reservoir 410
10 through the separation channel 412 by applying hydrostatic pressure to the reservoir 410 via
11 the entrance orifice 406.

12 Alternatively or additionally, the fluid may be driven through the separation channel
13 412 by applying a suitable electrokinetic potential voltage difference between the reservoir
14 electrode 426 and the exit electrode 428 to generate electrophoretic or electroosmotic fluidic
15 motion. Preferably, the electric potential difference is approximately 1000 V/cm of
16 separation channel length. Of course, any other suitable methods of inducing fluidic motion
17 may be utilized. Pressure-driven and voltage-driven flow effect different separation
18 efficiencies. Thus, depending upon the application, one or both may be utilized.

19 Fluid then exits from the separation channel 412 through the exit orifice 414 to, for
20 example, a capillary 434, which has an off-chip interconnection with the exit orifice 414, as
21 shown in FIG. 26. Alternatively, as shown in FIG. 27, the liquid chromatography device 400
22 may perform separation on the fluid from reservoir 410 such that selected analytes from the
23 separation performed by posts 416 passes through unpopulated channel 436 to another on-
24 chip device 438, such as for analysis and/or mixing, while the remainder of the fluid is
25 directed to the waste reservoir 439. The unpopulated channel 436 may be a mere
26 continuation of the separation channel 412 of the liquid chromatography device 400 or a
channel separate from the separation channel 412.

Two or more fluid samples may be driven through the liquid chromatography device
400 by successively filling the reservoir and driving the fluid through the separation channel
412. For example, in certain applications, it may be desirable or necessary to first coat the
surfaces of the separation posts 416 with one or more reagents and then pass an analyte
sample over the conditioned separation posts 416.

Various modifications may be made to the liquid chromatography device describe
above. For example, as shown in FIG. 28, rather than defining the entrance orifice and the

1 introduction channel in the substrate, the liquid chromatography device 400' may provide
2 an introduction channel 404' in the cover 420' such that the entrance orifice 406' is defined
3 on an exterior surface of the cover 420'. Further, the cover 420' may define an exit channel
4 413 between an exit orifice 414' defined on an exterior surface of the cover 420' and a
separation channel 412' which terminates within the substrate 402'.

5 In another variation, an additional introduction channel 440 and entrance orifice 442
6 may be defined in the substrate 402'', as shown in FIG. 29, or in the cover (not shown). The
7 additional introduction channel 440 introduces fluid to the separation channel 412'' such that
8 the fluid from the additional introduction channel 440 intersects the path of fluid flow from
9 the reservoir 410 through the unpopulated portion 432'' of the separation channel 412''. The
10 fluid reservoir 410 may be utilized as a buffer for an eluent and the additional introduction
11 channel 440 may be utilized to introduce the fluid sample to the separation channel 412''.
12 Further, the additional entrance orifice 442 may be utilized to introduce several fluid samples
13 in succession into the separation channel 412''. For example, in certain applications, it may
14 be necessary to first coat the surfaces of the separation posts 416 with one reagent and then
15 pass an analyte over the conditioned surfaces of the separation posts 416.

16 Referring now to FIGS. 30-35, although the liquid chromatography device has been
17 described as comprising a single reservoir and a single separation channel, the monolithic
18 liquid chromatography device may be easily adapted and modified to comprise multiples of
19 the liquid chromatography device and/or multiple entrance orifices, exit orifices, reservoirs
20 and/or separation channels. In each of the variations, any or all of the reservoir(s), separation
21 channel(s), and separation posts may have different dimensions and/or shapes.

22 For example, multiple reservoir-separation channel combinations may be provided
23 on a single chip. In particular, as shown in FIG. 30, a reservoir 410A may feed into a
24 separation channel 412A having separation posts 416A and another reservoir 410B may feed
25 into another separation channel 412B having separation posts 416B.

26 In another variation as shown in FIG. 31, a single reservoir 410C may feed multiple
separation channels 412C, 412D. Each of separation channels 412C, 412D may have therein
separation posts 416C, 416D, respectively, which may have the same or different properties,
such as number, size and shape. Another channel 412E may be provided as a null channel
completely unpopulated by separation posts. The output from the null channel 412E may
be utilized as a basis of comparison to the output from the separation channel(s) populated
by separation posts. Alternatively, all of the channels 412C, 412D, 412E may be separation
channels having separation posts.

1 Referring now to FIG. 32, fluid from multiple reservoirs **410E** and **410F** may feed
2 into a single separation channel **412F** via connecting channels **444E**, **444F**, respectively. The
3 connecting channels **444E**, **444F** are preferably unpopulated by separation posts to facilitate
4 the mixing of the fluid samples from the reservoirs **410E**, **410F** prior to passage through the
5 separation channel **412F**. The mixing of samples may be utilized to condition the primary
6 sample of interest prior to separation or to effect a reaction between the samples prior to
7 passage through the populated portion of the separation channel **412F**. Alternatively, fluid
8 such as a conditioning fluid from one reservoir **410E** may flow through the separation
9 channel **412F** in order to condition the surfaces of the separation posts **416F** prior to the
10 passage of the other sample such as an analyte sample from the other reservoir **410F**.
11 Although the separation posts **416F** are shown as having different cross-sections, separation
12 posts **416F** may have the same size and cross-sectional shape.

13 Alternatively, in addition to having fluid from multiple reservoirs feed into a single
14 separation channel via connecting channels, fluid from another reservoir may be introduced
15 to the fluid flow along the separation channel, before and/or after the fluid has passed
16 through the populated portion of the separation channel. For example, FIG. 33 shows that
17 the fluid from multiple reservoirs **410G**, **410H** may be fed into a single separation channel
18 **412G** via connecting channels **444G**, **444H**, respectively, and fluid from another reservoir
19 **410I** may be introduced to the fluid flow along the separation channel **412G** after the fluid
20 has passed the separation posts **416G**. FIG. 34 shows that the fluid from multiple reservoirs
21 **410J**, **410K** may be fed into a single separation channel **412J** via connecting channels **444J**,
22 **444K**, respectively, and fluid from another reservoir **410L** may be introduced to the fluid
23 flow along the separation channel **412J** prior to the fluid passing the separation posts **416J**.

24 For devices having multiple reservoirs and/or multiple channels, separate electrodes
25 may be provided for each reservoir and/or for each channel, for example, in the unpopulated
26 portion of the channel upstream from the separation posts and/or near the exit of the channel.
Such provision of separate electrodes allow for the separate and independent control of the
fluidic flow for filling each reservoir and/or for driving the fluid through the separation
channel.

The electric control may be simplified by having one common reservoir electrode,
one common filling electrode, and/or one exit electrode among the multiple reservoirs and/or
multiple channels. For example, each of the multiple reservoirs may be separately filled by
applying a first voltage to the common reservoir electrode and a second voltage, different
from the first voltage, to the filling electrode corresponding to the reservoir to be filled while

1 applying the first voltage to each of the other filling electrodes. As is evident, the multiple
2 reservoirs may be simultaneously filled by applying a first voltage to the common reservoir
3 electrode and a second, different voltage to each of the filling electrodes. Similarly, fluid
4 may be separately driven through each of the multiple channels by applying a third voltage
5 to the common reservoir electrode while applying a fourth voltage, different from the third
6 voltage, to the exit electrode corresponding to the channel through which fluid is to be driven
7 and the third voltage to each of the other exit electrodes.

8 In yet another variation shown in FIG. 35, in addition to a sample reservoir **410M**
9 and separation posts **416M**, a plurality of posts **416L** may be provided in a channel **412M**
10 upstream from the separation posts **416M** for providing additional functionality such as
11 solid-phase extraction (SPE) for sample pretreatment. The SPE posts **416L** may be the same,
12 similar to or different from the separation posts **416M** simply by varying the layout design.
13 The SPE posts **416L** may provide surface functionality different from that of the separation
14 posts **416M**. Alternatively, rather than providing a sample reservoir, an introduction channel
15 (not shown) may be utilized to introduce a fluidic sample directly in the channel **412M** by
16 allowing direct injection of the sample therein. Further, reservoirs **410N**, **410P** may be
17 provided to contain fluidic buffers necessary for sample pretreatment upstream of the posts
18 **416L**. For example, an eluent reservoir may be provided for eluting analytes and a wash
19 reservoir may be provided for sample cleanup.

20 After the fluid samples pass the SPE posts **416L**, waste products from, for example,
21 the solid-phase extraction process may be directed into a waste reservoir **410Q**. In particular,
22 during the SPE process, voltage differences may be applied between or amongst reservoirs
23 **410M**, **410N**, **410P**, and **410Q** such that a portion of the fluid from reservoirs **410M**, **410N**
24 is directed to waste reservoir **410Q** while the remaining portion of the fluid from reservoir
25 **410M** remain on the SPE posts **416L**. Material may then be washed off of the SPE posts
26 **416L** by directing fluid from, for example, reservoir **410P** through channel **412M** for
separation of the extracted material by separation posts **416M**. Additional reservoirs **410R**,
410S downstream of the waste reservoir **410Q** and upstream of the separation posts **416M**
may be provided to contain gradient elution of analytes in one reservoir and a diluent in the
other reservoir. Gradient elution facilitates chromatography by changing the mobile phase
composition, i.e. the polarity to facilitate analyte interactions with the stationary phase, and
thus facilitate separation of the analytes. In addition, the diluent provides the correct polarity
of the solution for the next separation.

LIQUID CHROMATOGRAPHY DEVICE FABRICATION PROCEDURE

The fabrication of the liquid chromatography device of the present invention will now be explained with reference to FIGS. 36A-46B. The liquid chromatography device is preferably fabricated as a monolithic silicon micro device utilizing established, well-controlled thin-film silicon processing techniques such as thermal oxidation, photolithography, reactive-ion etching (RIE), ion implantation, and metal deposition. Fabrication using such silicon processing techniques facilitates massively parallel processing of similar devices, is time- and cost-efficient, allows for tighter control of critical dimensions, is easily reproducible, and results in a wholly integral device, thereby eliminating any assembly requirements. Manipulation of separate components and/or sub-assemblies to build an liquid chromatography device with high reliability and yield is not desirable and may not be possible at the micrometer dimensions required for efficient separation.

Further, the fabrication sequence may be easily extended to create physical aspects or features to facilitate interfacing, integration and/or connection with devices having other functionalities or to facilitate integration with a fluid delivery subsystem to create a single integrated system. Consequently, the liquid chromatography device may be fabricated and utilized as a disposable device, thereby eliminating the need for column regeneration and eliminating the risks of sample cross-contamination.

Referring to the plan and cross-sectional views, respectively, of FIGS. 36A and 36B, a silicon wafer separation substrate **500**, double-side polished and approximately 250-600 μm in thickness, is subjected to an elevated temperature in an oxidizing ambient to grow a layer or film of silicon dioxide **502** on the reservoir side **503** and a layer or film of silicon dioxide **504** on the back side **505** of the separation substrate **500**. Each of the resulting silicon dioxide layers **502**, **504** has a thickness of approximately 1-2 μm . The silicon dioxide layers **502**, **504** provide electrical isolation and also serve as masks for subsequent selective etching of certain areas of the separation substrate **500**.

A film of positive-working photoresist **506** is deposited on the silicon dioxide layer **502** on the reservoir side **503** of the separation substrate **500**. Certain areas of the photoresist **506** corresponding to the reservoir, separation channel and separation posts which will be subsequently etched are selectively exposed through a mask by an optical lithographic exposure tool passing short-wavelength light, such as blue or near-ultraviolet at wavelengths of 365, 405, or 436 nanometers.

1 Referring to the plan and cross-sectional views, respectively, of FIGS. 37A and 37B,
2 after development of the photoresist **506**, the exposed areas **508**, **509**, **510** of the photoresist
3 corresponding to the reservoir, separation posts and channel, respectively, are removed and
4 open to the underlying silicon dioxide layer **502** while the unexposed areas remain protected
5 by photoresist **506'**. The exposed areas **508**, **509**, **510** of the silicon dioxide layer **502** are
6 then etched by a fluorine-based plasma with a high degree of anisotropy and selectivity to
7 the protective photoresist **506'** until the silicon separation substrate **500** is reached. The
8 remaining photoresist is removed in an oxygen plasma or in an actively oxidizing chemical
9 bath like sulfuric acid (H_2SO_4) activated with hydrogen peroxide (H_2O_2).

10 As shown in the cross-sectional view of FIG. 38, the reservoir **410**, the separation
11 channel **412**, and the separation posts **416** in the separation channel **412** are vertically formed
12 in the silicon separation substrate **500** by another fluorine-based etch. Preferably, the
13 reservoir **410** and the separation channel **412** have the same depth controlled by the etch time
14 at a known etch rate. The simultaneous formation of the reservoir **410** and the channel **412**
15 ensures uniform depth such that there are no discontinuities in the fluid-constraining surfaces
16 to impede the fluid flow. The depth of the reservoir **410** and the channel **412** is preferably
17 between approximately 5-20 μm and more preferably approximately 10 μm . The etch can
18 reliably and reproducibly be executed to produce an aspect ratio (etch depth to width) of up
19 to 30:1. Although not shown, any other reservoirs and/or channels, populated or
20 unpopulated, may also be formed by this etch sequence.

21 A film of positive-working photoresist is then deposited over the silicon dioxide
22 layer **502** and the exposed separation substrate **500** on the reservoir side **503** of the separation
23 substrate **500**. An area of the photoresist corresponding to the introduction channel which
24 will be subsequently etched is selectively exposed through a mask by an optical lithographic
25 exposure tool passing short-wavelength light, such as blue or near-ultraviolet at wavelengths
26 of 365, 405, or 436 nanometers. After development of the photoresist, the exposed area of
the photoresist corresponding to the introduction channel is removed and open to the
underlying separation substrate **500** while the unexposed areas remain protected by the
photoresist.

As shown in the plan and cross-sectional views of FIGS. 39A and 39B, respectively,
the exposed area of the separation substrate **500** is then vertically etched by a fluorine-based
plasma with a high degree of anisotropy and selectivity to the protective photoresist until the
silicon dioxide layer **504** on back side **505** is reached. Thus, a portion of the introduction
channel **404** is formed through the separation substrate **500**. The remaining photoresist is

1 removed in an oxygen plasma or in an actively oxidizing chemical bath like sulfuric acid
2 (H_2SO_4) activated with hydrogen peroxide (H_2O_2). The silicon dioxide layer **504** on the back
3 side **505** may then be removed by, for example, an unpatterned etch in a fluorine-based
4 plasma.

5 Alternatively, as shown in FIGS. 40A and 40B, the introduction channel **404** may
6 be formed by etching from both the reservoir side **503** and the back side **505** of the substrate
7 **500**. After performing a vertical etch through a portion of the substrate **500** to form a portion
8 of the introduction channel **404** in a manner similar to that described above, a film of
9 positive-working photoresist **512** is deposited on the silicon dioxide layer **504** on the back
10 side **505** of the separation substrate **500**. Patterns on the back side **505** may be aligned to
11 those previously formed on the reservoir side **503** of the separation substrate **500**. Because
12 silicon and its oxide are inherently relatively transparent to light in the infrared wavelength
13 range of the spectrum, i.e. approximately 700-1000 nanometers, the extant pattern on the
14 reservoir side **503** can be distinguished with sufficient clarity by illuminating the separation
15 substrate **500** from the patterned reservoir side **503** with infrared light. Thus, the mask for
16 the back side **505** can be aligned within required tolerances. Upon alignment, an area of the
17 photoresist **512** corresponding to the entrance orifice and the introduction channel which will
18 be subsequently etched is selectively exposed through a mask by an optical lithographic
19 exposure tool passing short-wavelength light, such as blue or near-ultraviolet at wavelengths
20 of 365, 405, or 436 nanometers.

21 After development of the photoresist **512**, the exposed area **514** of the photoresist
22 corresponding to the entrance orifice is removed to expose the underlying silicon dioxide
23 layer **504** on the back side **505** of the separation substrate **500** while the unexposed areas
24 remain protected by the photoresist **512**. The exposed area **514** of the silicon dioxide layer
25 **504** is then etched by a fluorine-based plasma with a high degree of anisotropy and
26 selectivity to the protective photoresist **512** until the substrate **500** is reached. The remaining
photoresist provides additional masking during a subsequent fluorine-based silicon etch to
vertically etch the backside portion of the introduction channel. Thus, a through-substrate
introduction channel **404** is complete. The remaining photoresist is removed in an oxygen
plasma or in an actively oxidizing chemical bath like sulfuric acid (H_2SO_4) activated with
hydrogen peroxide (H_2O_2).

Preferably, the introduction channel **404** has the same diameter as the entrance
orifice. A practical limit on etch aspect ratio of 30:1 constrains the diameter of the entrance
orifice being etched to be approximately 10 μm or greater for substrates of approximately

1 300 μm thickness. Preferably, the entrance orifice 406 and the introduction channel 404
2 are approximately 100 μm in diameter due to practical considerations. For example, the
3 etch aspect ratio imposes a minimum diameter, and the diameter is preferably sufficiently
4 large to enable ease of filling the reservoir 410 yet sufficiently small to ensure a fluid surface
5 tension to prevent the fluid from leaking out of the reservoir 410.

6 Alternatively, both the introduction channel and the entrance orifice may be formed
7 by etching from the back side 505 of the separation substrate 500. This may be preferable
8 as it may be difficult to satisfactorily coat the separation posts 416 with photoresist. Further,
9 this may be desirable depending on the application of the device, e.g. the external sample
10 delivery system, the desired chip handling devices, the interfacing with other devices, chip-
11 based or non-chip based, and/or the packaging considerations of the chip. Referring to the
12 cross-sectional view of FIG. 41, after the reservoir, separation channel and the separation
13 posts are etched in the separation substrate 500 (shown in FIG. 38), a film of positive-
14 working photoresist 516 is deposited on the silicon dioxide layer 504 on the back side 505
15 of the separation substrate 500. Patterns on the back side 505 may be aligned to those
16 previously formed on the reservoir side 503 of the separation substrate 500 by illuminating
17 the separation substrate 500 from the patterned reservoir side 503 with infrared light, as
18 described above. Upon alignment, an area of the photoresist 516 corresponding to the
19 entrance orifice which will be subsequently etched is selectively exposed through a mask by
20 an optical lithographic exposure tool passing short-wavelength light, such as blue or near-
21 ultraviolet at wavelengths of 365, 405, or 436 nanometers.

22 After development of the photoresist 516, the exposed area 518 of the photoresist
23 516 corresponding to the entrance orifice is removed to expose the underlying silicon dioxide
24 layer 504 on the back side 505 of the separation substrate 500. The exposed area 518 of the
25 silicon dioxide layer 504 is then etched by a fluorine-based plasma with a high degree of
26 anisotropy and selectivity to the protective photoresist 512 until the silicon separation
substrate 500 is reached. The remaining photoresist is left in place to provide additional
masking during the subsequent etch through the silicon separation substrate 500.

Referring now to the cross-sectional view of FIG. 42, the introduction channel 404
is vertically formed through the silicon separation substrate 500 by another fluorine-based
etch. The introduction channel 404 is completed by etching through the separation substrate
500 until the reservoir 410 is reached. Thus, the introduction channel 404 extends through
the separation substrate 500 between the entrance orifice 406 on the back side 505 of the
separation substrate 500 and the reservoir 410. The remaining photoresist is removed in an

1 oxygen plasma or in an actively oxidizing chemical bath like sulfuric acid (H_2SO_4) activated
2 with hydrogen peroxide (H_2O_2).

3 **Oxidation for surface passivation and fluid isolation**

4 As shown in the cross-sectional view of FIG. 43, a layer of silicon dioxide **522** is
5 grown on all silicon surfaces of the substrate **500** by subjecting the silicon substrate **500** to
6 elevated temperature in an oxidizing ambient. For example, the oxidizing ambient may be
7 an ultra-pure steam produced by oxidation of hydrogen for a silicon dioxide thickness greater
8 than approximately several hundred nanometers or pure oxygen for a silicon dioxide
9 thickness of approximately several hundred nanometers or less. The layer of silicon dioxide
10 **522** over all silicon surfaces of the separation substrate **500** electrically isolates a fluid in the
11 channel from the silicon substrate **500** and permits the application and sustenance of an
12 electric potential difference between the reservoir and the exit of the separation channel,
13 between the reservoir and an unpopulated portion of the separation channel near the reservoir
14 to facilitate in filling the reservoir and/or between other points along the fluid flow path.
15 Thus, the application and sustenance of a significant voltage across the fluid sample may be
16 achieved. Further, oxidation renders a surface inactive relative to a bare silicon surface,
17 resulting in surface passivation.

18 All silicon surfaces are oxidized to form silicon dioxide with a thickness that is
19 controllable through choice of temperature and time of oxidation. The final thickness of the
20 silicon dioxide can be selected to provide the desired degree of electrical isolation in the
21 device, where a thicker layer of silicon dioxide provides a greater resistance to electrical
22 breakdown.

23 Photolithography and reactive-ion etching limit the layout design of separation post
24 diameters and inter-post spacing to greater than approximately $1\ \mu m$. However, because the
25 thermal oxidation process consumes approximately $0.44\ \mu m$ of silicon to form each
26 micrometer of silicon dioxide, the thermal oxidation process results in a volumetric
expansion. This volumetric expansion may be utilized to reduce the spacing between the
separation posts **416** to sub-micrometer dimensions. For example, with a layout inter-post
spacing of approximately $1.5\ \mu m$, oxidation producing a $1\ \mu m$ silicon dioxide film or layer
would result in a nearest-neighbor spacing of approximately $0.5\ \mu m$. Further, because the
oxidation process is well-controlled, separation post dimensions, including the inter-post
spacing, in the sub-micrometer regime can be formed reproducibly and in a high yielding
manner.

1 FIGS. 44A, 44B and 44C show scanning electron microscope photographs and
2 design layout of portions of fabricated liquid chromatography devices. FIG. 44A shows a
3 design layout of a portion of a reservoir and separation posts in a portion of a separation
4 channel where the separation posts have rectangular cross-sectional shape. FIG. 44B shows
5 separation posts in a portion of a separation channel, the separation posts having a circular
6 cross-sectional shape and a diameter and inter-post spacing of approximately 1 μm . FIG. 44C
7 shows separation posts in a portion of a separation channel, the separation posts having a
8 rectangular or square cross-sectional shape with a dimension of 2 μm and inter-post spacing
9 of approximately 1 μm .

10 In a variation, the entrance orifice and the introduction channel for filling the fluid
11 reservoir may be formed in the cover substrate 524 after a layer of silicon dioxide 525 is
12 grown on all surfaces of the cover substrate 524, rather than in the substrate 500. As shown
13 in FIG. 45, the cover substrate 524 may be bonded to the reservoir side 503 of the separation
14 substrate 500. The entrance orifice 406' and the introduction channel 404' may be formed
15 in the cover substrate 524 after alignment with respect to the reservoir 410. The entrance
16 orifice 406' and the introduction channel 404' may be formed in the same or similar manner
17 as described above by utilizing lithography to define the entrance orifice pattern and reactive-
18 ion etching to create the entrance orifice and the through-cover introduction channel. The
19 cover substrate 524 is again subjected to elevated temperature in an oxidizing ambient to
20 grow a layer of oxide on the surface of the introduction channel 404'. Further, the
21 introduction channel 404' may be formed from one or two sides of the cover substrate 524.
22 If channel 404' is formed from two sides of the cover substrate, the cover substrate 524 may
23 be bonded to substrate 500 after forming the channel 404' and after oxidation of the channel
24 surface. One advantage of defining the entrance orifice on the same side of the completed
25 liquid chromatography device as the reservoir and separation channel is that the back side
26 of the substrate 500 is then free from any features and may then be bonded to a protective
package without special provision for filling the reservoir through an entrance orifice defined
on the back-side of the substrate.

Metallization for fluid flow control

FIGS. 46A and 46B illustrate the formation of a reservoir, a filling, and an exit
electrode as well as conductive lines or wires connecting the electrodes to bond pads in the
cover substrate 526, preferably comprising glass and/or silicon. The cover substrate 526
shown in FIGS. 46A and 46B does not provide an entrance orifice or an introduction channel

1 although the metallization process described herein may be easily adapted for a cover
2 substrate providing an entrance orifice and an introduction channel.

3 As shown in the plan and cross-sectional view of FIGS. 46A and 46B, respectively,
4 prior to the depositing of conductive material on the cover substrate **526**, all surfaces of the
5 cover substrate **526** are subjected to thermal oxidization in a manner that is the same as or
6 similar to the process described above to create a film or layer of silicon dioxide **528**. Such
7 oxidization is not performed where the cover substrate **526** comprises glass.

8 The silicon dioxide layer **528** provides a surface on which conductive electrodes may
9 be formed. The thickness of the silicon dioxide layer **528** is controllable through the
10 oxidation temperature and time and the final thickness can be selected to provide the desired
11 degree of electrical isolation, where a thicker layer of silicon dioxide provides a greater
12 resistance to electrical breakdown. The silicon dioxide layer **528** electrically isolates all
13 electrodes from the cover substrate **526** and isolates the fluid in the reservoir and the channel
14 of the liquid chromatography device from the cover substrate **526**. The ability to isolate the
15 fluid from the cover substrate **526** complements the electrical isolation provided in the
16 separation substrate through oxidation and ensures the complete electrical isolation of the
17 fluid from both the separation substrate and the cover substrate **526**. The complete electrical
18 isolation of the sample fluid from both substrates allows for the application of electric
19 potential differences between spatially separated locations in the fluidic flow path resulting
20 in control of the fluid flow through the path.

21 The cover substrate **528** may be cleaned after oxidation utilizing an oxidizing
22 solution such as an actively oxidizing chemical bath, for example, sulfuric acid (H_2SO_4)
23 activated with hydrogen peroxide (H_2O_2). The cover substrate **528** is then thoroughly rinsed
24 to eliminate organic contaminants and particulates. A layer of conductive material **530** such
25 as aluminum is then deposited by any suitable method such as by DC magnetron sputtering
26 in an argon ambient. The thickness of the aluminum is preferably approximately 3000 Å,
although shown having a larger thickness for clarity. Although aluminum is utilized in the
fabrication sequence described herein, any type of highly conductive material such as other
metals, metallic multi-layers, silicides, conductive polymers, and conductive ceramics like
indium tin oxide (ITO) may be utilized for the electrodes. The surface preparation for
satisfactory adhesion may vary depending on the specific electrode material used. For
example, the silicon dioxide layer **528** provides a surface to which aluminum electrodes may
adhere as aluminum does not generally adhere well to native silicon.

1 A film of positive-working photoresist **532** is then deposited over the surface of the
2 conductive material **530**. Areas of the photoresist layer **532** corresponding to areas
3 surrounding the electrodes (shown) and conductive lines or wires and bond pads which will
4 be subsequently etched are selectively exposed through a mask by an optical lithographic
5 exposure tool passing short-wavelength light, such as blue or near-ultraviolet at wavelengths

6 After development of the photoresist **532**, the exposed areas of the photoresist are
7 removed, leaving opening to the underlying aluminum conductive layer **530** while the
8 unexposed areas **534**, **536**, **538** corresponding to the reservoir, filling and exit electrodes,
9 respectively, as well as conductive lines or wires and bond pads remain protected by the
10 photoresist. The conductive electrodes and the lines/bond pads may be etched, such as by
11 a wet chemical etch or a reactive-ion etch, as appropriate for the particular conductive
12 material. The etch is selective to the underlying silicon dioxide layer **528** or is terminated
13 upon reaching the silicon dioxide layer **528** as determined by the etch time and rate. The
14 remaining photoresist is removed in an oxygen plasma or in a solvent bath such as acetone.
15 The fabrication sequence thus results in physically and electrically separate islands of
16 conductive electrodes, lines and bond pads according to the pattern designed in the mask.

17 The cover substrate may be larger than the separation substrate to allow access to
18 the bond pads and/or directly to the electrodes for the application of potential voltage(s) to
19 the electrode(s). As shown in FIG. 46C, the cover substrate **526'** is larger than the separation
20 substrate such that the separation substrate only extends to dashed line **540** relative to the
21 cover substrate **526'**. Conductive lead-throughs such as connecting metal lines **542**, **544** and
22 **546** extend from the reservoir, filling and exit electrodes, **534**, **536**, **538**, respectively, and
23 enable the application of potential voltage(s) to the electrode(s).

24 Alternatively, a metal lead may be formed from each electrode to an otherwise
25 unpatterned area of the separation substrate such that a through-substrate access channel
26 formed in the cover substrate and filled with a conductive material by chemical vapor
deposition (CVD) allows access to the electrode(s). As an alternative to chemical vapor
deposition, the sidewalls of the through-substrate access channel may be sloped, for example
by KOH etch, to facilitate continuous deposition of a conductive material thereon, thereby
providing an electrically continuous path from the separation substrate to the top of the cover
substrate where potential voltages can be applied. In these variations, the separation and the
cover substrates may be of the same size.

1 Although the electrodes are preferably provided on a surface of the cover substrate,
2 the electrodes may be alternatively and/or additionally provided on the separation substrate
3 by appropriate modifications to the above-described fabrication process. For example, in
4 such a variation, the side walls of the reservoir are preferably not at a 90° angle relative to
5 the bottom wall and can be formed at least in part by, for example, a wet chemical potassium
6 hydroxide (KOH) etch. The sloped reservoir side walls allow for the deposition of a
7 conductive material thereon. In another variation, the electrodes may also be formed by a
8 damascene process, known in the art of semiconductor fabrication. The damascene process
9 provides the advantage of a planar surface without the step up and step down surface
10 topography presented by a bond line or pad and thus facilitates the bonding of the separation
11 and cover substrate, as described below.

12 The above described fabrication sequence for the liquid chromatography device may
13 be easily adapted to and is applicable for the simultaneous fabrication of a monolithic system
14 comprising multiple liquid chromatography devices including multiple reservoirs and/or
15 multiple separation channels as described above embodied in a single monolithic substrate.

16 Further, although the fabrication sequence is described in terms of fabricating a
17 single liquid chromatography device, the fabrication sequence facilitates and allows for
18 massively parallel processing of similar devices. The multiple liquid chromatography
19 devices or systems fabricated by massively parallel processing on a single wafer may then
20 be cut or otherwise separated into multiple devices or systems.

21 Although control of the liquid chromatography device has been described above as
22 comprising reservoir, filling and exit electrodes, any suitable combination of such and/or
23 other electrodes in electrical contact with the fluid in the fluid path may be provided and
24 easily fabricated by modifying the layout design. Further, any or all of the electrodes may
25 be additionally or alternatively provided in the separation substrate. Electrodes may be
26 formed in the separation substrate by modifying the fabrication sequence to include
additional steps similar to or the same as the steps as described above with respect to the
formation of the electrodes in the cover substrate.

Bonding cover substrate to separation substrate

As described above, the cover substrate is preferably hermetically bonded by any
suitable method to the separation substrate for containment and isolation of the fluid in the
liquid chromatography device. Examples of bonding silicon to silicon or glass to silicon
include anodic bonding, sodium silicate bonding, eutectic bonding, and fusion bonding.

1 For example, to bond the separation substrate to a glass cover substrate by anodic
2 bonding, the separation substrate and cover substrate are heated to approximately 400°C and
3 a voltage of 400-1200 Volts is applied, with the separation substrate chosen as the anode (the
4 higher potential). Further, as the required bonding voltage depends on the surface oxide
5 thickness, it may be desirable to remove the oxide film or layer from the back side 505 of the
6 separation substrate prior to the bonding process in order to reduce the required bonding
7 voltage. The oxide film or layer may be removed by, for example, an unpatterned etch in a
8 fluorine-based plasma. The etch is continued until the entire oxide layer has been removed,
9 and the degree of over-etch is unimportant. Thus, the etch is easily controlled and high-
10 yielding.

11 Critical considerations in any of the bonding methods include the alignment of
12 features in the separation and the cover substrates to ensure proper functioning of the liquid
13 chromatography device after bonding and the provision in layout design for conductive lead-
14 throughs such as the bond pads and/or metal lines so that the electrodes (if any) are
15 accessible from outside the liquid chromatography device. Another critical consideration is
16 the topography created through the fabrication sequence which may compromise the ability
17 of the bonding method to hermetically seal the separation and cover substrates. For example,
18 the step up and step down in the surface topography presented by a metal line or pad may be
19 particularly difficult to form a seal therearound as the silicon or glass does not readily deform
20 to conform to the shape of the metal line or pad, leaving a void near the interface between
21 the metal and the oxide.

22 **INTEGRATION OF LIQUID CHROMATOGRAPHY AND ELECTROSPRAY** 23 **DEVICES ON A CHIP**

24 The cross-sectional schematic view of FIG. 47 shows a liquid chromatography-
25 electrospray system 600 comprising a liquid chromatography device 602 of the present
26 invention integrated with an electrospray device 620 of the present invention such that a
homogeneous interface is formed between the exit orifice 614 of the liquid chromatography
device 602 and the entrance orifice 622 of the electrospray device 620. The single integrated
system 600 allows for the fluid exiting the exit orifice 614 of the liquid chromatography
device 602 to be delivered on-chip to the entrance orifice 622 of the electrospray device 620
in order to generate an electrospray.

As shown in FIG. 47, the entrance orifice 606 and the introduction channel 604

1 of the liquid chromatography device 602 are formed in the cover substrate 608 along with
2 the electrospray device 620. Alternatively, the liquid chromatography entrance orifice and
3 the introduction channel may be formed in the separation substrate.

4 Fluid at the electrospray nozzle entrance 622 is at the exit voltage applied to the exit
5 electrode 610 in the separation channel 612 near the liquid chromatography exit orifice 614.
6 Thus, an electrospray entrance electrode is not necessary.

7 The single integrated system 600 provides the advantage of minimizing or
8 eliminating extra fluid volume to reduce the risk of undesired fluid changes, such as by
9 reactions and/or mixing. The single integrated system 600 also provides the advantage of
10 eliminating the need for unreliable handling and attachment of components at the
11 microscopic level and of minimizing or eliminating fluid leakage by containing the fluid
12 within one integrated system.

13 The integrated liquid chromatography-electrospray system 600 may be utilized to
14 deliver liquid samples to the sampling orifice of a mass spectrometer. The sampling orifice
15 of the mass spectrometer may serve as an extraction electrode in the electrospray process
16 when held at an appropriate voltage relative to the voltage of the electrospray nozzle 624.
17 The liquid chromatography-electrospray system 600 may be positioned within 10 mm of the
18 sampling orifice of the mass spectrometer for efficient extraction of the fluid from the
19 electrospray nozzle 624.

20 **Multiple liquid chromatography-electrospray systems on a single chip**

21 Multiples of the liquid chromatography-electrospray system 600 may be formed on
22 a single chip to deliver a multiplicity of samples to a common point for subsequent
23 sequential analysis. For example, FIG. 48 shows a plan view of multiple liquid
24 chromatography-electrospray systems 600 on a single chip 650 and FIG. 49 shows a detailed
25 view of area A of systems 600 with the separation channels shown in phantom and without
26 the recessed portions for purposes of clarity. As shown, the multiple nozzles 624 of the
electrospray devices 620 may be radially positioned about a circle having a relatively small
diameter near the center of the single chip 650. The dimensions of the electrospray nozzles
and the liquid chromatography channels limit the radius at which multiple nozzles are
positioned on the multi-system chip 650. For example, the multi-system chip may provide
96 nozzles with widths of up to 50 μm positioned around a circle 2 mm in diameter such that
the spacing between each pair of nozzles is approximately 65 μm .

Alternatively, an array of multiple electrospray devices without liquid
chromatography devices may be formed on a single chip to deliver a multiplicity of

1 samples to a common point for subsequent sequential analysis. The nozzles may be similarly
2 radially positioned about a circle having a relatively small diameter near the center of the
3 chip. The array of electrospray devices on a single microchip may be integrated upstream
4 with multiple fluid delivery devices such as separation devices fabricated on a single
5 microchip. For example, an array of radially distributed exit orifices of a radially distributed
6 array of micro liquid chromatography columns may be integrated with radially distributed
7 entrance orifices of electrospray devices such that the nozzles are arranged at a small radius
8 near the orifice of a mass spectrometer. Thus, the electrospray devices may be utilized for
9 rapid sequential analysis of multiple sample fluids. However, depending upon the specific
10 application and/or the capabilities of the downstream mass spectrometer (or other
11 downstream device), the multiples of the electrospray devices may be utilized one at a time
or simultaneously, either all or a portion of the electrospray devices, to generate one or more
electrosprays. In other words, the multiples of the electrospray devices may be operated in
parallel, staggered or individually.

The single multi-system chip 650 may be fabricated entirely in silicon substrates,
thereby taking advantage of well-developed silicon processing techniques described above.
Such processing techniques allow the single multi-system chip 650 to be fabricated in a cost-
effective manner, resulting in a cost performance that is consistent with use as a disposable
device to eliminate cross-sample contamination. Furthermore, because the dimensions and
positions of the liquid chromatography-electrospray systems are determined through layout
design rather than through processing, the layout design may be easily adapted to fabricate
multiple liquid chromatography-electrospray systems on a single chip.

Interface of a multi-system chip to mass spectrometer

The radially distributed array of electrospray nozzles 624 on a multi-system chip
may be interfaced with a sampling orifice of a mass spectrometer by positioning the nozzles
near the sampling orifice. The tight radial configuration of the electrospray nozzles 624
allows the positioning thereof in close proximity to the sampling orifice of a mass
spectrometer.

The multi-system chip 650 may be rotated relative to the sampling orifice to position
one or more of the nozzles for electrospray near the sampling orifice. Appropriate voltage(s)
may then be applied to the one or more of the nozzles for electrospray. Alternatively, the
multi-system chip 650 may be fixed relative to the sampling orifice of a mass spectrometer
such that all nozzles, which converge in a relatively tight radius, are appropriately
positioned for the electrospray process. As is evident, eliminating the need for nozzle

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repositioning allows for highly reproducible and quick alignment of the single multi-system chip and increases the speed of the analyses.

One, some or all of the radially distributed nozzles **624** of the electrospray devices **620** may generate electrosprays simultaneously, sequentially or randomly as controlled by the voltages applied to the appropriate electrodes of the electrospray device **620**.

While specific and preferred embodiments of the invention have been described and illustrated herein, it will be appreciated that modifications can be made without departing from the spirit of the invention as found in the appended claims.

What is claimed and desired to be secured by United States Letters Patent is: